FINAL REPORT

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INTEGRATED DISEASE MANAGEMENT FOR CHICKPEA, LENTIL AND FIELD PEA

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Integrated Disease Management for Chickpea, Lentil and Field Pea

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Executive Summary

In 1997, the contract for this project was signed in late spring 1997. Drs. Buchwaldt and Chongo were hired, Zeneca Agro agreed to contribute $40,000, fungicide trials were set out at 13 sites across the prairies, draft versions of the decision support systems (DDS) for management of foliar diseases in lentil and pea were developed, and several studies were initiated.

In 1998, two M.Sc. students were recruited; they started in May. The DDSs for lentil and pea were assessed at multiple locations. Studies verified that both mating types of *Ascochyta rabiei* (cause of ascochyta blight in chickpea) occurred at sites across the prairies. Field studies showed a substantial decline in survival of *A. rabiei* inoculum after 2-3 years of rotation with non-host crops. A range of new studies on pathogenic diversity in *Colletotrichum truncatum*, sources of foliar disease resistance in chickpea and lentil, survival of *A. rabiei* in infected residue, factors affecting infection and resistance to ascochyta blight in chickpea, impact and management of root rots and seedling blights, etc., were initiated.

In 1999, fungicide demonstration trials on pea, chickpea and lentil at AFIF Spoke sites were added to the ongoing studies for the DSS conducted in commercial fields and AAFC research centres. Data from 22 tests was used to evaluate the DDS for lentil; the system consistently provided good recommendations. A DSS for mycosphaerella blight of field pea was also assessed. It worked well for some cultivars, but not for others, e.g. cv. Carneval and Highlight. Carneval developed severe blight when disease pressure was high, and application of fungicide reduced blight severity but did not improve yield. The sexual stage of *A. rabiei* was identified for the first time in western Canada. Air-borne ascospores produced by the sexual stage can be carried over long distances by the wind, and are an important source of primary disease inoculum in some years. The existence of two races of *C. truncatum* on lentil was confirmed, and the geographic distribution of the races in western Canada was assessed. Field trials demonstrated that seedling blight and root rot diseases of pulses could have an important impact on stand establishment and yield in western Canada, with the main pathogens being *Fusarium* spp. on lentil, *Pythium* spp on field pea, and *Rhizoctonia solani* on chickpea. Management strategies for seedling blight were assessed at several locations. Cooperative studies with scientists across the region showed that a wide range of crops, including pulses, could act as hosts for *Fusarium graminearum*, the pathogen that causes fusarium head blight of cereals.

In 2000, field trials and AFIF spoke trials on the DSS for pea and lentil and on fungicide management of ascochyta blight on chickpea were completed. The DSS for lentil provided correct recommendations in 35 of 40 possible tests, and was recommended to growers. The DSS for field pea did not provide consistent predictions and was withdrawn. However, this study showed that assessing blight tolerance in pea lines represents a much more promising approach for plant breeders than a continued search for resistance. In both greenhouse and field tests, resistance in ascochyta-resistant chickpea cultivars adapted for western Canada was high in young plants, but declined quickly at flowering. This supported observations from the fungicide trials, which showed that fungicide application at late flowering was often highly effective. Fungicide application routinely increased chickpea yields by more than 50%, and growers were quick to adopt this technology. Data from the study was used to support an emergency registration for Quadris fungicide on chickpea in 2000 and 2001, as well as an application for regular registration on all three pulse crops. Field studies showed that seedling blight on field pea

were much more severe when seed was cracked or damaged during handling, and that fungicide seed treatments provided effective disease management.

Isolates of *A. rabiei* (40 from Saskatchewan, 18 from other countries) were assessed for pathogenicity on eight chickpea lines under controlled conditions. These eight lines were selected for use as differentials from an initial pool of 62 resistant lines from around the world. Large differences in pathogenicity were noted among the isolates, and 15 pathotypes were identified. These groupings demonstrate that the population is highly variable, and that many different pathogenic races of *A. rabiei* are present in Canada. To further assess the genetic diversity of the pathogen population, these same isolates were assessed using RAPDs. Isolates from Canada encompassed all of the genetic diversity observed in isolates from the other countries. There was no correlation between RAPD groups and pathotype groupings.

The process of infection by *C. truncatum* in lentil was studied on one partially resistant and one susceptible lentil line, using one highly aggressive and one moderately aggressive isolate. Conidia germinated within 3-6 hr post inoculation (hpi), and formed appressoria at 6-12 hpi. Hyphae in host tissues grew inter- and intra-cellularly. In the partially-resistant line, hyphal aggregations were prominent for the moderately-aggressive isolate, but were not produced by the highly-aggressive isolate. No aggregations were observed in the susceptible line. Also, hyphal spread was slower in the resistant line, resulting in smaller lesions than in the susceptible line.

In 2001, a long-term field study and a long-term study under partially-controlled conditions confirmed our earlier observations that *A. rabiei* can survive for at least three years in infected stubble, and that three years between successive chickpea crops is required to reduce pathogen populations to acceptable levels.

Throughout the study, a strong emphasis was placed on getting the new research information out to extension specialists, producers and other scientists as quickly as possible. A website on "Pulse crop diseases" was launched in May 1999. The site included descriptions of disease symptoms, disease management options, decision support systems, and a map of real-time information on disease outbreaks across the province during the growing season. Information on integrated disease management in pulse crops was presented at numerous extension meetings during the winter and in various extension publications, reports and scientific papers. So far, scientists in the project have published 11 scientific paper (+ 4 in review), 21 reports, 24 abstracts, 21 tech transfer articles, a M.Sc. thesis, an invited conference presentation and a book chapter, with more still in preparation.

We feel that the objectives of the study have not only been met, but exceeded in every respect, and that this project has contributed significantly to the continued success of pulse production, especially of chickpea, in western Canada.

Technical Report

Background

Pulse crops are an important component of agricultural production in western Canada. For example, the value of pea, lentil, chickpea, and bean production in Saskatchewan for 1995 was over $396 million. Pulse crops are also important in crop diversification, and have a major impact on farm income when cereal prices are low. Producers also profit from; 1) almost no nitrogen fertilizer requirement for the pulse crop, 2) reduced nitrogen requirements for the following crop, 3) increased protein content of the following grain crop, and 4) reduced losses from disease, insect and weed pests from improved crop rotation.

High quality and stable production levels for pulses are required to stay competitive in global markets. Foliar diseases are the most important threat to the quality and quantity of pulses produced in western Canada, so disease management is critical to maintaining our market share. Evaluation of foliar fungicides for control of disease in lentil and pea have been carried out in western Canada in recent years, and product efficacy has been demonstrated. However, fungicide application is expensive and may not be required every year. Growers need information on the conditions under which fungicide application will be cost-effective.

Chickpea acreage is rapidly increasing. Diseases, especially ascochyta blight caused by *Ascochyta rabiei*, result in severe losses in seed quality and yield, even in partially-resistant cultivars. Disease management is possible using a combination of resistant cultivars and foliar fungicides. However, many races of *A. rabiei* have been identified world-wide. Existing systems to identify races are inadequate, so it is impossible to determine which races are present in a seed lot, a field, or even a country. However, this information is of vital importance to plant breeders working to develop resistant cultivars. Furthermore, little is known about the impact of management techniques such as tillage and crop rotation on the long-term survival of *A. rabiei* in soil and crop residue.

Objectives

1. Develop a decision support system for fungicidal control of ascochyta blight and anthracnose of lentil, and ascochyta blight of pea, and make the system available to producers.

2. Develop a cost-effective package of recommendations for control of ascochyta blight of chickpea [*Ascochyta rabiei*].

3. Identify races of *Ascochyta rabiei* in Canada.

Project Activities and Achievements

Objective 1. DECISION SUPPORT SYSTEMS FOR DISEASE MANAGEMENT

Background: Draft versions of decision support systems for foliar fungicide application to manage foliar diseases on lentil (ascochyta blight and anthracnose) and pea (mycosphaerella blight) were developed at the start of the project. The systems are empirically based upon: 1) severity of disease symptoms (disease thresholds) before and during flowering, 2) frequency of rain in the past 14 days, 3) the 5-day weather forecast, and 4) plant stand. The effects of crop rotation, tillage practices, seed-borne infection, wind-borne inoculum and similar factors are reflected in the level of disease symptoms. Data for verification of the systems were gathered in 1997-2000 from trials in Saskatchewan (lentil), and across the prairie region (field pea). The objectives were: 1) to test a fungicide decision support system, 2) to compare rates and time of application of chlorothalonil and azoxystrobin.

Fungicide trials - Lentil

Materials and Methods: Many of the tests in this study were conducted in grower's fields. In addition, small plot trials were conducted at seven AFIF spoke sites across Saskatchewan in 1999 and 2000 (reported separately). The tests in grower's fields all followed the same protocol, with minor modifications among sites and years. Each trial was arranged in a randomized complete block design with four replications. Each plot was 2.4 x 6 m. At early flowering, 1.2 m walkways were rotor-tilled to establish plots in an area of the field with uniform plant stand and low weed pressure. The treatments were: an unsprayed control, a single application of Bravo 500 at 1.0 kg a.i. ha^{-1} and Quadris at 125 g a.i. ha^{-1} applied at early flowering, at mid flowering, or at early + mid flowering. The fungicides were applied by a CO_2 sprayer (generally a bicycle sprayer) fitted with TeeJet 8003 nozzles spaced 0.5 m apart, at 275 kPa in at least 200 L of water per hectare. Disease ratings were made prior to each fungicide application, and about 3 wk after the final application. The seed samples were dried, cleaned and weighed. Seed samples from 10 trials were graded according to the Canadian Grain Commissions Guidelines 1999, based on a bulked sample comprised 25 g of each of the four reps per treatment and site. Percentages of foreign material, stained and damaged seed determined the seed grade as follows: No. 1= 0.2%, 1.0 %, and 2.0 %; No. 2= 0.5%, 4.0 %, and 3.5 %; Extra No. 3= 0.5%, 7.0 %, and 5.0 %; No. 3= 1.0%, unlimited stained, and 10.0 % damaged seed; and Grade= if No.3 specifications were not met. There was no deduction for stained seed in cultivars with red cotyledons, such as CDC Redwing, since they are dehulled and split. The General Linear Models Procedure (SAS) was used to analyse the disease severity and yield data.

In 1997, trials were set out at three commercial lentil fields and one research farm site in Saskatchewan. In 1998, trials were established in commercial lentil crops at four sites in Saskatchewan. Fields at Tessier, Zealandia and Elrose were planted to cv. Laird and a second field at Elrose was planted to cv Crimson. In addition, two new formulations of Bravo, Ultrex and IB10353 at 1.68 a.i. ha^{-1}, were compared to Bravo 500 at early flowering. The first fungicide

applications were made between July 8-13, with the second 10-14 days later. A rating of anthracnose was made at the two spray dates. Anthracnose was also rated 1 wk before harvest by assessing the severity (on a 0-3 scale) of 10-15 plants per plot and calculating the percent disease severity index (% DSI) for each plot as shown below. The crops were desiccated with Roundup between August 5 and 10, except cv. Laird in Elrose, which dried out without desiccation and was harvested on August 20. The other trials were direct combined 10-14 days after dessication.

In 1999, field trials were established in two commercial fields of lentil cv. Laird at Zealandia and Sovereign SK. There were 12 treatments per site. Fungicides were applied on July 6 and 20 at Zealandia and on July 14 and 29 at Sovereign. Severity of anthracnose and ascochyta blight on leaves and stems was assessed at five sites per plot using the Horsfall-Barrett scale (0-11), and converted to % plant area affected. The trial at Sovereign was harvested on August 12. The site at Zealandia was inadvertently destroyed by the cooperator before harvest. In 2000, trials were established at Bladworth and Zealandia in commercial fields of cv. Laird.

In addition, fungicide trials on lentil were established at 6 of 7 AFIF spoke sites in 1999 and 2000. The treatments were generally similar to those reported above, and they were managed as part of this project, but the results have been reported as part of an AFIF spoke project.

Results and Discussion: In 1997, hot, dry weather inhibited foliar disease development at most sites, so foliar fungicides had little or no effect on yield (data not shown). In 1998, anthracnose developed slowly in cv. Laird at Zealandia; severity was 18% in the unsprayed control at harvest (Table 1). Necrotic lesions were present on lower leaflets and a premature leaf drop was evident, indicating that fungicide application might be economically beneficial. A t Elrose, anthracnose developed rapidly, reaching 57% in the control at harvest (Table 1). Both of these fields were planted to lentils for the first time, so the primary infection was most likely caused by wind-borne inoculum. As the season progressed, patches of heavily infected plants became evident, especially in cv Laird at Elrose. However, no foliar diseases were observed in cv Laird at Tessier or cv. Crimson at Elrose. There were no consistent differences among the treatments across locations in disease severity or seed yield (Table 1).

In 1999, above-normal rainfall in July and August resulted in high levels of anthracnose and ascochyta blight. Leaf lesions and premature leaf drop caused by anthracnose were evident in all plots at both Sovereign and Zealandia (2-4%) at the date of the first fungicide application. At the second date of application, anthracnose had increased in the untreated control plots to 21% in Sovereign and 13% in Zealandia. By the third rating date, the disease was severe at both sites (90% at Sovereign, 77% at Zealandia). Sclerotinia stem rot and botrytis grey mold occurred sporadically, but were not included in the disease ratings. A single application of a low rate of Quadris (125 g a.i. ha^{-1}) at early flower reduced severity of anthracnose and ascochyta and increased yield (Table 2) more than a single application of Bravo. Two applications of Quadris at the low rate did not improve yield over a single application (Table 2). There were no differences between two applications of Bravo, of Quadris, or both fungicides. The time of application seems to be less critical with Quadris than with Bravo which is an advantage since it allows more time for field scouting and better disease diagnosis. Quadris had a adverse effect on yield when applied at the high rate (175 g a.i. ha^{-1}) at early flower, but no evidence of phytotoxicity was observed.

In 2000, disease severity was high at both sites. Anthracnose was the dominant pathogen at

Zealandia. Fungicide application reduced anthracnose severity, but only the early application of Quadris resulted in increased seed yield. At Bladworth, ascochyta was the main pathogen, but grey mold came in late in the season. All of the fungicide treatments reduced disease severity and increased yield at this site.

Seed grade was assessed on samples from 10 sites. Grade varied as much between plots with the same treatment as among different treatments, so an average seed grade was determined for each treatment. At Redvers (2000), where anthracnose severity was 85-90% at harvest, one application of Quadris at mid flower increased the seed grade from No.3 to Extra No. 3. The same application in cv. Redwing at Outlook (1999) increased the seed grade from Extra No. 3 to No. 2, but not in cv. Richlea where all treatments were No.2. At Scott (2000) and Outlook (2000), where grey mould was the dominant disease, one application of Bravo at early flower increased the seed grade from No. 3 to Extra No.3. At five sites, there was no improvement from fungicide treatment of the following seed grades: Scott (1999) No.2, Swift Current (2000) Extra No. 3, Sovereign (1999) Extra No.3, Zealandia (2000) No. 3, and Bladworth (2000) Grade. None of the samples were No.1. Some of the low grades were due to a large amount of broken, peeled and split seed, as a result of handling by the plot-combine and dry harvest conditions, and so could not be attributed to diseases (data not shown). Infrequent improvement of seed grade did not allow its inclusion in calculation of economic return from fungicide application.

Table 1. Effect of Bravo and Quadris applied at different times and rates (g a.i. ha^{-1}) on anthracnose severity (% leaf area affected) and seed yield in four commercial lentil crops in Saskatchewan, 1998.

Fungicide, timing, rate/ha			Tessier	Zealandia		Elrose		Elrose
			Laird	cv. Laird		Laird		Crimson
Fungicide	Early flower	Mid flower	Yield Mg/ha	Sever-ity (%)	Yield Mg/ha	Sever-ity (%)	Yield Mg/ha	Yield Mg/ha
Control			0.95	18	1.01	57	0.95	0.89
Bravo	1.0 kg		0.98	31	1.05	51	0.92	0.89
Bravo		1.0 kg	0.84	18	1.16	46	0.87	0.81
Bravo	1.0 kg	1.0 kg	0.93	31	0.96	50	0.97	0.9
Quadris	125 g		1.09	20	1.00	52	0.95	1.04
Quadris		125 g	0.87	18	1.13	56	0.96	0.94
Quadris	125 g	125 g	0.93	20	1.07	58	1.12	0.93
Bravo	1.7 kg		0.92	6	1.14	49	0.86	1.06
Bravo Ultrex	1.7 kg		1.07	13	0.94	57	0.94	0.98
Bravo IB10353	1.7 kg		0.93	25	1.00	48	0.96	1.02
F-test			ns		ns		ns	ns

Table 2. Effect of Bravo and Quadris on severity[1] of anthracnose and ascochyta blight in lentil at two locations in Saskatchewan, 1999.

Fungicide, Timing, Rate/ha			Zealandia	Sovereign	
Fungicide	Early flower	Mid flower	% Severity July 6, 20,Aug 19	% Severity July 14, 29, Aug 19	Yield Mg/ha
1. Control			2 / 13 / 77	4 / 21 / 90	0.59 e
2. Bravo	1.0 kg		2 / 7 / 42	6 / 12 / 57	1.13 bcd
3. Bravo		1.0 kg	2 / 9 / 40	5 / 20 / 70	0.93 cde
4. Bravo	1.5 kg		1 / 7 / 50	4 / 24 / 30	1.26 abc
5. Bravo	1.0 kg	1.0 kg	1 / 5 / 26	2 / 21 / 23	1.44 ab
6. Quadris	125 g		1 / 6 / 59	4 / 18 / 44	1.41 ab
7. Quadris		125 g	2 / 6 / 25	4 / 24 / 50	1.40 ab
8. Quadris	175 g		1 / 5 / 62	4 / 26 / 66	0.83 de
9. Quadris	125 g	125 g	1 / 5 / 51	3 / 13 / 15	1.58 a
10. Bravo ULTREX	1.0 kg		1 / 7 / 37	4 / 31 / 57	1.12 bcd
11. Bravo + Quadris	1.0 kg	125 g	2 / 5 / 30	4 / 19 / 28	1.29 abc
12. Quadris + Bravo	125	1.0 kg	2 / 5 / 44	4 / 23 / 16	1.43 ab
$LSD_{0.05}$			1 / 5 / 28	3 / 14 / 24	0.39

[1] % leaf and stem area infected by anthracnose and ascochyta blight at the first and second date of fungicide application, and 3-4 weeks after the last application.

Decision Support System - Lentil

Background: Repeated requests from growers for information on when to apply foliar fungicides to control the foliar diseases of lentil was the force that drove this portion of the project. This strong demand also prompted us to make the decision support system available to as many growers as possible, even during the evaluation phase of the project. It has been available for two years on the Pulse Crop Diseases web site, in a factsheet "Management of Lentil Anthracnose," in articles in grower magazines and was presented at numerous extension meetings. Our objective was to develop and verify a decision support system for lentil that was applicable for both anthracnose and ascochyta blight. The system was tested over years using a registered fungicide (Bravo) and a potential new fungicide (Quadris).

Materials and Methods: Results from fungicide trials (from the present studies and previous reports) and empirical observations were used to design a decision support system (DSS) to identify the circumstances under which application of foliar fungicide in lentil would reduce injury caused by ascochyta blight and anthracnose, increase seed yield and quality, and increase net returns. The following four factors were included in the DSS (Table 3): A) severity of anthracnose and ascochyta blight symptoms between the 10-12 node stage and mid flower; B) number of days with rain in the previous 14 days; C) likelihood of rain in the next 5-day period, based on weather forecasts; and D) density of the plant stand. Risk points between 0 and 25 were assigned to each of these factors. The DSS relied upon inspection of the crop for symptoms of anthracnose and ascochyta blight starting at the 10-12 nod stage, and ideally repeated every 3-5 days. At each inspection date, the risk value that most closely described the current situation was determined for each of the four risk factors, and the total risk value was calculated as the sum of A+B+C+D, resulting in a value between 0 and 75. Reliability of the fungicide decision support system was tested at 16 locations between 1998 and 2000, using six research station sites in Saskatchewan and six commercial lentil crops (described previously). At four of these research station sites, two cultivars were assessed each year. The DSS was evaluated on five cultivars; Crimson, CDC Glamis, Laird, and CDC Richlea (susceptible to anthracnose and ascochyta blight), and CDC Redwing (susceptible to anthracnose, partially resistant to ascochyta blight). Infection relied upon natural inoculum, except at Redvers and Canora where anthracnose-infected lentil residue was spread in the plots after planting in 2000. Risk values were calculated in each trial at both early and mid flower, thereby allowing the fungicide decision support system to be evaluated in 40 cases. Approximately 120 plants m^{-2} was regarded as a normal plant stand, 75-120 moderate, <75 thin, and >120 plants m^{-2} dense. If a 5-day weather forecast could not be obtained, a risk value was assigned to the actual number of days with rain in the 5 day period following fungicide application as: 0 days = 0, 1-2 days = 5, 3-4 days = 10, and 5 days = 15.

Between 4 and 6 wk after the last fungicide application was made, the severity of foliar diseases caused by *C. truncatum*, *A. lentis*, *B. cinerea*, and *S. sclerotiorum* was determined. A rating of affected leaf and stem area was made using a 0-11 scale and converted to percentages. Mean values were calculated for each treatment. The DDS was rated as successful when a risk value above the threshold of 50 coincided with a significant yield increase from the fungicide treatment, or when a value less than 50 was assigned at a site where no yield increase occurred.

Results: In 24 of 40 cases, the DDS risk value was less than 50 at both early and mid flower, so a fungicide application was not recommended (Table 4). This negative recommendation was correct in 21 instances. However, in 3 of 24 cases (CDC Redwing and CDC Richlea at Scott in 1999, CDC Glamis at Outlook in 2000), yield was higher after a fungicide application that in the nonsprayed control.

In 16 of 40 cases, the threshold value of 50 was reached at either early or mid flower (Table 5). In 14 cases, fungicide application resulted in a significant yield increase. Anthracnose was the major disease at 5 sites. The DSS correctly indicated the need for fungicide application at 2 sites where grey mold symptoms occurred in stands with high plant density under wet weather conditions (Bladworth and Scott in 2000). However, the threshold value was not reached until mid flower at 2 sites (CDC Redwing at Outlook in 1999, CDC Glamis at Redvers in 2000), while the optimum time of application was at early flower, based on subsequent yield. This error in timing could have been avoided if the plots were inspected at 3-5 days intervals and treated accordingly, but the research plan only allowed fungicides application with 10-14 days interval. Overall, of the 40 cases in the evaluation, there were 35 correct and 5 incorrect risk assessments.

Seed grading was conducted on samples from 10 of the trials, but differences were generally small and inconsistent. These data were highly variable because of large number of bruised or split seeds. This damage had nothing to do with the impact of disease on seed quality, and little relevance for producers using large-scale commercial harvesting equipment, so the differences were not included in the DSS evaluation.

Discussion: The DSS provide the correct recommendation (spray/don't spray) in 88% (35 of 40) of all cases. In the 3 cases where a fungicide application increased yield when the risk value was below 50 (Table 4), the impact of the fungicide application may have been due to protection from late-season disease development. The fungicide effect at Scott may also have been due to the exceptionally dense plant stand. At the two sites where moderate levels of anthracnose triggered a fungicide application but no yield differences were found (Zealandia in 1998, Elrose in 1998, Table 5), the trials were quite variable because of very patchy distribution of infection and dry weather conditions, so a yield increase would have been hidden by this variability.

Premature leaf drop at early flowering, not associated with lack of light or nutrients, is an important indicator of anthracnose. It was therefore assigned a high risk value in the DSS. Grading of *A. lentis* symptoms was based on empirical knowledge that any amount of infection of flowers, petioles and peduncles should be assigned a high risk value in the DSS, as well as lesions on more than 10% of the upper and lower canopy (Table 3). Time of appearance and severity of these disease symptoms were intended to summarize the amount of inoculum from wind-, stubble- and seed-borne sources, along with variation caused by crop rotation, stubble management, and cultivar resistance, which are difficult or impossible to assess individually.

Conidia of both pathogens are spread by rainsplash, so the number of rain events was included to reflect the opportunity for secondary spread, and to allow for amount of latent infections. A dense plant stand would create a humid microclimate that is conducive for disease spread, but also enhances the crop's ability to compete with weeds, and increases yield potential, which would increase the likelihood of recovering the cost of a fungicide application compared to a thin, weed-infested plant stand. Since high plant density and frequent rain events promoted foliar disease severity and enhanced yield at the same time, both factors increased the need for

fungicide application and were assigned high risk values in the DSS.

Disease severity alone contributed a maximum of 50% of the risk points required to trigger a fungicide application (25 of 50 points), but inclusion of rain events and plant stand resulted in an acceptable level of accuracy. In the high-risk group, application of Quadris increased yield at 4 of 8 locations. Bravo increased yield at all 7 locations where it was assessed (not tested at early flower at Zealandia in 2000, which was the optimum time for treatment according the DSS). This indicates that Bravo provides more effective control of foliar diseases on lentil than Quadris. The cost of a single application of Bravo ranges between \$25-30 ha^{-1}, which corresponds to between 80 to 100 kg ha^{-1} yield increase depending on seed grade and market price. Significant yield increases ranged from 280 to 1770 kg ha^{-1} in trials conducted in 1992-93 (previous studies, not shown) and from 300 to 800 kg ha^{-1} in 1998-2000 (Table 5), well above the economic threshold of one or even several fungicide applications. Bravo was approved in1993 for use in lentil in Canada, and Quadris has received emergency registration on pulses in 2000 and 2001, and an application for full registration is in progress.

We believe that the fungicide decision support system is valid in the major lentil production area, across a range of crop management practices. The DSS was widely distributed to growers by Saskatchewan Agriculture and Food the Saskatchewan Pulse Growers organisation, agri-business companies, and on the web at htpp://paridss.usask.ca/specialcrop/pulse_diseases. More than 10,000 copies of an extension factsheet describing the system have been purchased (cost-recovery basis) by industry and other groups for distribution to their clients or members. Extension specialists from Saskatchewan Agriculture & Food took over maintenance of this website at the end of the project, where it continues to provide a valuable resource for growers. A scientific paper describing the development and validation of the DSS has been prepared and is currently in review prior to submission to a professional journal.

Table 3. A fungicide decision support system (DSS) for foliar fungicide application in lentil to control of anthracnose and ascochyta blight in western Canada.

Risk factors	Risk value
A. Symptoms of anthracnose and ascochyta blight	
No visible symptoms	0
Lesions on 1-5% of lower leaflets	5
Lesions on 6-10% of lower leaflets	15
Lesions on >10% of lower and upper leaflets	25
Premature drop of infected leaflets, and 1-5% of stems with small lesions	25
Flowers and peduncles infected	25
B. Number of days with rain in the last 14 days	
0 days	0
1-2 days	5
3-4 days	10
5-6 days	15
7 or more days	20
C. The 5-day weather forecast	
Dry	0
Unpredictable	5
Light showers	10
Rain	15
D. Plant density	
Thin (resulting in many weeds and low yield potential)	0
Moderate	5
Normal (approximately 120 plants m^{-2})	10
Dense canopy (more plants than normal or cultivar with lush growth habit)	15
Total risk value (A + B + C + D)	0 - 75

Table 4. Locations and lentil cultivars with risk values less than the threshold value of 50 at both early and mid flower, as calculated using the Decision Support System, in relation to disease severity near harvest, and seed yield in untreated and fungicide treated plots.

Disease, Location, Year	Fungicide treatment	Sev-erity (%)	Yield (Mg/ha)	Risk values		
				Risk factor	Early flower	Mid flower
Anthracnose at Elrose 1998		cv. Laird				
	Untreated	57	0.95			
	Bravo ef	51	0.92	A	5	25
	Bravo mf	46	0.87	B	20	0
	Quadris ef	52	0.95	C	0	0
	Quadris mf	56	0.96	D	10	10
	$LSD_{0.05}$		ns	Total	35	35
Anthracnose Zealandia 1998		Laird				
	Untreated	18	1.01			
	Bravo ef	31	1.05	A	5	25
	Bravo mf	18	1.16	B	10	0
	Quadris ef	20	1	C	0	0
	Quadris mf	18	1.13	D	10	10
	$LSD_{0.05}$		ns	Total	25	40
Ascochyta blight at Scott 1999		CDC Redwing				
	Untreated	11	2.23	A	5	5
	Bravo ef	7	2.5	B	15	5
	Quadris ef	7	2.39	C	0	10
	Quadris mf	6	2.77	D	15	15
	$LSD_{0.05}$		0.49	Total	35	35
		CDC Richlea				
	Untreated	17	1.91	A	5	5

	Bravo ef	10	2.9	B	15	5
	Quadris ef	9	2.33	C	0	10
	Quadris mf	6	2.03	D	15	15
	$LSD_{0.05}$		0.49	Total	35	35
Grey mold, sclerotinia at Outlook 2000		CDC Glamis				
	Untreated	10	1.8	A	0	5
	Bravo mf	5	2.24	B	20	5
	Quadris ef	15	1.68	C	5	5
	Quadris mf	7	1.83	D	10	10
	$LSD_{0.05}$		0.41	Total	35	25
Leaf spots at Swift Current 2000		CDC Glamis				
	Untreated	1	2.09	A	0	5
	Bravo mf	1	2.11	B	15	15
	Quadris ef	1	2.23	C	5	5
	Quadris mf	1	2.11	D	10	10
	$LSD_{0.05}$		ns	Total	30	35
Leaf spots at Swift Current 1999		CDC Redwing				
	Untreated	2	2.31	A	5	5
	Bravo ef	4	1.9	B	15	15
	Quadris ef	5	2.58	C	15	0
	Quadris mf	6	2.19	D	10	10
	$LSD_{0.05}$		ns	Total	45	30
		CDC Richlea				
	Untreated	2	2.07	A	5	5
	Bravo ef	5	2.08	B	15	15
	Quadris ef	4	2.13	C	15	0
	Quadris mf	3	1.91	D	10	10
	$LSD_{0.05}$		ns	Total	45	30

Leaf spots at Indian Head 1999		CDC Redwing				
	Untreated	6	1.22	A	5	5
	Bravo ef	8	1.23	B	15	10
	Quadris ef	6	1.2	C	0	0
	Quadris mf	7	1.11	D	10	10
	$LSD_{0.05}$		ns	Total	30	25
		CDC Richlea				
	Untreated	5	1.23	A	5	5
	Bravo ef	9	1.33	B	15	10
	Quadris ef	6	1.13	C	0	0
	Quadris mf	7	1.03	D	10	10
	$LSD_{0.05}$		ns	Total	30	25
No disease at Tessier 1998		cv. Laird				
	Untreated	0	0.96			
	Bravo ef	0	0.98	A	0	0
	Bravo mf	0	0.84	B	10	0
	Quadris ef	0	1.09	C	0	0
	Quadris mf	0	0.87	D	10	10
	$LSD_{0.05}$		ns	Total	20	10
No disease at Elrose 1998		cv. Crimson				
	Untreated	0	0.89			
	Bravo ef	0	0.89	A	0	0
	Bravo mf	0	0.81	B	20	0
	Quadris ef	0	1.04	C	0	0
	Quadris mf	0	0.94	D	10	10
	$LSD_{0.05}$		ns	Total	30	10

[1] See text for a detailed explanation of risk categories and risk values
[2] Bravo at 1 kg a.i. ha^{-1} at early (ef) or mid (mf) flower.
[3] Quadris at 125 g a.i. ha^{-1} at early or mid flower.

Table 5. Seven locations (8 lentil cultivars) with risk values higher than the threshold value of 50 at either early or mid flower as calculated by the Decision Support System, in relation to disease severity at harvest, and seed yield in untreated and fungicide treated plots.

Disease, Location, Year	Fungicide treatment	Sev-erity (%)	Yield (Mg/ha)	Risk factor	Risk values Early flower	Risk values Mid flower
Anthracnose at Outlook 1999			CDC Redwing			
	Untreated	90	1.97 b	A	5	25
	Bravo ef	83	2.28 a	B	20	15
	Quadris ef	95	1.84 b	C	0	5
	Quadris mf	84	2.02 b	D	5	5
	$LSD_{0.05}$		0.22	Total	30	50
			CDC Richlea			
	Untreated	30	2.67 c	A	5	25
	Bravo ef	16	2.85 b	B	20	15
	Quadris ef	22	2.56 c	C	0	5
	Quadris mf	21	3.11 a	D	5	5
	$LSD_{0.05}$		0.22	Total	30	50
Anthracnose at Canora 2000 (inoculated)			CDC Glamis			
	Untreated	25	1.19 b	A	15	15
	Bravo ef	18	1.64 a	B	20	15
	Quadris ef	35	1.15 b	C	10	10
	Quadris mf	11	0.99 b	D	10	10
	$LSD_{0.05}$		0.39	Total	55	50
Anthracnose at Redvers 2000 (inoculated)			CDC Glamis			
	Untreated	85	1.11 b	A	5	25
	Bravo mf	45	1.24 b	B	15	15
	Quadris ef	55	1.48 a	C	10	5
	Quadris mf	30	1.20 b	D	5	5
	$LSD_{0.05}$		0.26	Total	35	50

Anthracnose at Zealandia 2000		Laird				
	Untreated	70	1.21 b	A	25	25
	Bravo mf	17	1.26 b	B	15	10
	Quadris ef	9	1.57 a	C	15	0
	Quadris mf	6	1.28 b	D	10	10
	$LSD_{0.05}$		0.27	Total	65	45
Anthracnose & Ascochyta at Sovereign 1999		Laird				
	Untreated	90	0.59 b			
	Bravo ef	57	1.13 a	A	25	25
	Bravo mf	70	0.93 b	B	10	15
	Quadris ef	44	1.41 a	C	5	10
	Quadris mf	50	1.40 a	D	10	10
	$LSD_{0.05}$		0.39	Total	50	60
Grey mold & Ascochyta at Bladworth 2000		Laird				
	Untreated	85	0.82 b			
	Bravo ef	35	1.07 b	A	5	25
	Bravo mf	nd	1.29 a	B	15	15
	Quadris ef	24	1.07 b	C	5	5
	Quadris mf	15	1.07 b	D	10	10
	$LSD_{0.95}$		0.42	Total	35	55
Grey mold at Scott 2000		CDC Glamis				
	Untreated	6	1.58 b	A	5	5
	Bravo mf	3	2.12 a	B	20	20
	Quadris ef	7	1.65 b	C	10	10
	Quadris mf	4	1.87 ab	D	15	15
	$LSD_{0.05}$		0.38	Total	50	50

[1] A = Severity of anthracnose and ascochyta, B = Number of rain days in the past 14 days, C = Risk of rain in the 5-day weather forecast, D = Density of plant stand.
[2] Bravo at 1 kg a.i. ha^{-1} or Quadris at 125 g a.i. ha^{-1} at early (ef) or mid (mf) flower.

Fungicide trials - Field pea

Background: Field pea is an important component of the crop rotations in Saskatchewan, especially in the dark-brown and black soil regions. Mycosphaerella blight, caused by *Mycosphaerella pinodes*, is the dominant pathogen of pea. In most years, it occurs on every plant in every field in the region. Yield losses have been reported to range as high as 50%, and the air-borne nature of the primary inoculum (ascospores) ensures that crop management has little impact on disease levels in individual fields (e.g. Bailey, K.L., Johnston, A.M., Kutcher, H.R., Gossen, B.D. and Morrall, R.A.A. 2000. Managing crop losses from foliar diseases with fungicides, rotation, and tillage in the Saskatchewan Parkland. Can. J. Plant Sci. 80: 169-175. Reprint in Appendix 1). No resistant cultivars have been identified, so application of foliar fungicides is one of the few options available for disease control. Several fungicides were recently registered for this purpose, but have not been tested across the region. Also, new actives have recently become available, which may represent new opportunities for disease management.

Method: All of these trials used minor modifications from the same basic research protocol that was used for lentil. In 1997 and 1998, a split-plot design was used with two pea cultivars as main-plot and fungicide treatment as sub-plots. There were seven fungicide treatments: an unsprayed control, Bravo 500 (50% a.i. chlorothalonil) at 1.0 and 1.5 kg a.i. ha^{-1} and Quadris (22.9% w/w azoxystrobin) at 125 and 175 g a.i. ha^{-1} each applied at early flower; and two applications of Bravo or Quadris applied at early and late flower. Foliar severity was rated as leaf area affected at the bottom, middle and top of the pea canopy at five sites per plot, and a mean value for each of the three canopy levels were calculated for each plot. Severity was rated prior to each fungicide application, and 3 wk after the last fungicide application. Stem disease severity was rated on a 0-9 scale just before harvest. Powdery mildew was rated in the last week of July. The plots were direct combined, and the seed dried, cleaned and weighed. The general linear models procedure of SAS was used to analyse the data.

In 1997, trials were set out at 7 sites in western Canada (2 in SK, 4 in AB and 1 in MB). The trials were all seeded trials at research centres, where they could be used as demonstration sites for producers attending the station field days. Disease severity was assessed using a 0-9 scale, where disease was assessed at slight, moderate or severe at each level of the canopy (e.g. 1 = slight in the lower canopy, 8 = moderate in the upper canopy).

In 1998, five trials were established at Melfort, Lacombe and Vegreville, AB, and Morden MB research farms, using the basic protocol, with cvs Carneval (upright growth habit) and Highlight (resistant to powdery mildew).

In 1999, trials with a single cultivar were established at research farms at Westlock and Mundare AB, and in commercial fields at Star City (cv. Carneval) and Prince Albert SK (cv. Alfetta). A new formulation of Bravo was added to the trial, so there were 12 fungicide treatments, using the experimental design and management described previously. In 1999, the disease assessment was modified slightly, to a % leaf area affected assessment at each level in the canopy.

In 2000, trials were set out at Westlock, Mundare, Prince Albert (cv. Delta) and Star City. Dithane (mancozeb) was added to the trial, so each trial included 13 treatments plus an unsprayed control in a randomized complete block design with four replications.

In addition, fungicide trials on field pea were established at 7 AFIF spoke sites in 1999 and 2000. The treatments were generally similar to those reported here, and they were managed as

part of this project, but the results were reported as part of an AFIF spoke project.

Results and Discussion: In 1997, hot, dry weather inhibited foliar disease development at most sites, so foliar fungicides had little or no effect on yield.

In 1998, there was a large variation in plant establishment between and within locations. Plants m^{-2} ranged between 30-70 in Melfort, 50-110 in Star City, and 60-120 in Morden (no count in Vegreville). As expected, powdery mildew did not develop on the resistant cv Highlight, but on cv Carneval the powdery mildew severities were 5.5 in Morden, 3.5 in Vegreville, 0.7 in Melfort and 2.0 on cv Keoma in Star City. None of the fungicide treatments on cv Highlight resulted in significant yield differences, so only data from cvs Carneval and Keoma are reported (Table 6). Also, the trial at Lacombe was damaged by pigeons (seed yield not shown). In Morden, the first foliar rating was higher (2.5) than at the other locations. Weather conditions remained conducive for disease development, and severity was 3.6 on the second date of application, and 4.6 in the final rating on the unsprayed control. The blight epidemic started early and was relatively severe, so the highest seed yield was obtained with a single early application of Bravo at the high rate. Severity in Vegreville was low at the first date of fungicide application (1.0), but high on the second date (3.4), and reached 4.8 in the unsprayed control 3 wk later. As a result, the highest seed yield was obtained with two applications of either Bravo or Quadris (Table 7). At Star City, severity was low at the first (1.0) and second (2.2) applications dates, but developed to 5.2 at the final rating date. In this case, an application of Quadris at early flower significantly increased yield. At Melfort, disease severity was low at both application dates (1.0, 2.0) and the disease did not develop further, so yield was not improved by fungicide treatment and there were no differences among treatments. The absence of measurable effect of fungicide treatment in cv Highlight was not explained.

At Mundare and Westlock, both fungicide applications at early flower and 10-14 days later, were made at zero or trace levels of mycosphaerella blight. Three to four weeks after the last fungicide application between 20-50% of the bottom 1/3 of the canopy was infected at the trial Mundare, while less than 20% was affected at Westlock (data not shown). The disease did not spread to the middle or top of the canopy at neither location. One treatment, the early low rate application of Quadris at Mundare, significantly increased yield, while the rest were not significantly different from the control (Table 7). This could be an artifact, since this treatment in combination with other fungicide treatments did not increase yield as would be expected. Furthermore, yield increase from fungicide application would not be expected at these low levels of mycosphaerella blight.

In 1999 at Star City, mycosphaerella blight severity was about 1-2% infection on the first date of application and 10% on the second date (data not shown). On August 4, the disease had increased to almost 100% at the bottom of the canopy, 25-50% in the middle, and 1-20 % at the top (Table 8). Only small yield increases resulted from fungicide application; probably because both applications were made too early, when infections were still low. This is supported by the fact that a fungicide applied at mid flower resulted in higher yields than at early flower, although these differences were not statistically significant. At Prince Albert, there was 0.5-4% infection of mycosphaerella at the bottom of the canopy on the first date of application and 15% on the second date (data not shown). On August 23, severity had increased to almost 100% at the bottom 1/3 of the canopy, 30-80% in the middle, and 1-30% at the top (Table 8). Some of the Bravo and Quadris treatments reduced disease severity in the middle and top of the canopy. At this high disease pressure, Quadris by itself or in combination with Bravo increased yield above

the untreated control. Quadris at 175 g applied at early flower did not adversely affect yield, as it did in lentil and chickpea. There was no difference between the liquid formulation of chlorothalonil (Bravo 500) and the granular formulation (Bravo ULTREX), but neither treatment increased yield. Quadris followed by Bravo tended to increase yield more than Bravo followed by Quadris, but differences were not statistically significant. There was a stronger yield response to fungicide in the susceptible cultivar Alfetta (Prince Albert) than on Carneval (Star City), but earlier planting and flowering of the pea trial in Star City might also have had an effect.

In 2000 at Star City, severity was very low early during the flowering period (1-2% of the pea leaf area affected on the first date of application, and 10% on the second date). By the final rating date, mycosphaerella blight had spread to almost 100% in the lower canopy, 25-50% in the middle, and 1-20 % at the top (Table 9). Only small yield increases resulted from fungicide application; probably because both applications were made when infections were still low. This is supported by the observation that yields tended to be higher when the fungicide was applied at mid flower than at early flower, although these differences were not statistically different. At Prince Albert, blight severity was 0.5-4% in the lower canopy on the first date of application and 15% on the second date. On August 23, mycosphaerella blight had increased to almost 100% in the lower canopy, 30-80% in the middle, and 1-30% at the top (Table 9). Some Bravo and Quadris treatments reduced disease levels in the middle and top of the canopy. Quadris, by itself or in combination with Bravo, increased yield compared to the control. Quadris did not affect yield when applied at 175 g a.i. ha^{-1} applied at early flower. There was no difference between the liquid formulation of chlorothalonil, the granular formulation, and the control. When Quadris was followed by an application of Bravo, yields tended to be higher than when Quadris followed Bravo, but the difference was not statistically significant.

Blight severity was low at the Prince Albert site at the first and second fungicide application dates, with only 30-40% of the bottom canopy was affected and less than 2% in the middle canopy. This low level of disease was associated with cool conditions during May and June, which delayed pathogen development and spread, even though precipitation was adequate. On August 14, the disease had spread to 40-50% in the bottom canopy, 30% in the middle, and 10-20% at the top. With increased temperatures in July and frequent showers, blight developed more rapidly toward the end of the growing season.

None of the fungicide treatments resulted in a yield increase over the unsprayed control (Table 9). This was likely that due to the late onset of disease as a result of the cool weather conditions. The crop was well into flower and pod development before infection levels developed on the middle and upper foliage. The frequent rainfall resulted in high pea yields and may have compensated for damaged caused by mycosphaerella blight. Under these conditions there were no difference between the liquid (Bravo 500) and granular (Bravo Ultrex) formulation of chlorothalonil.

Conclusion: Results across years and locations in this 4-yr, multi-site study indicate that seed yield of field pea was not generally affected by mycosphaerella blight. For example, fungicide application increase yield at only 1 of 7 sites in 2000. Analysis of data from 23 trials across the prairies showed that fungicide treatment in pea was rarely profitable due to a combination of several factors such as tolerance to mycosphaerella blight in some cultivars, late onset of disease, early harvest, variable yield potential and high fungicide costs.

Table 6. Effect of fungicide treatment in pea on mycosphaerella blight severity three weeks after the last fungicide application (S3) and stem disease severity rated before harvest (SDS) in 1998.

	Early	Mid	Melfort SK		Star City SK		Vegreville AB		Morden MB	
Fungicide	flower	flower	S3	SDS	S3	SDS	S3	SDS	S3	SDS
Unsprayed			2.0	4.8 a	5.2	5.2 a	4.8 a	5.2 bcd	4.6 a	5.8 a
Bravo	1000[1]		2.0	4.5 a	4.0	2.5 bc	3.2 b	6.1 ab	4.3 ab	5.4 ab
Bravo	1500		2.0	5.0 a	3.5	4.7 ab	3.1 b	5.8 abc	3.8 c	4.9 bc
Bravo	1000	1000	2.0	3.5 b	4.5	2.0 c	3.0 b	5.0 cd	3.9 bc	4.8 bc
Quadris	125		2.0	5.0 a	3.5	3.5 abc	3.3 b	6.3 a	4.2 abc	4.8 c
Quadris	175		2.0	4.5 a	4.2	2.5 bc	3.0 b	5.1 cd	3.9 bc	4.6 bc
Quadris	125	125	2.0	4.8 a	4.0	1.5 c	3.3 b	4.4 d	4.3 ab	4.3 c
$LSD_{0.05}$			ns	0.8	ns	2.6	0.6	1.0	0.5	0.7

1) gram active ingredient per hectare

Table 7. Effect of foliar fungicide, timing and rate on seed yield (Mg ha^{-1}) and relative yield (% of unsprayed control = Rel.) of field pea at four sites in 1998.

	Early	Mid	Melfort SK		Star City SK		Vegreville AB		Morden MB	
Fungicide	flower	flower	Mg/ha	Rel.	Mg/ha	Rel.	Mg/ha	Rel.	Mg/ha	Rel.
Unsprayed			1.96	100	2.50	100	3.09	100	6.08	100
Bravo	1000[1]		2.06	105	3.03	121	3.44	111	6.42	105
Bravo	1500		2.19	111	2.70	108	3.59	116	6.55	107
Bravo	1000	1000	2.17	111	2.99	120	3.90	126	6.32	104
Quadris	125		1.79	91	3.21	128	3.36	108	6.07	100
Quadris	175		2.17	111	2.90	116	3.43	111	5.95	98
Quadris	125	125	1.86	95	2.95	118	4.00	129	6.19	102
$LSD_{0.05}$			ns		0.58		0.71		ns	

1) gram active ingredient per hectare

Table 8. Effect of Bravo 500 and Quadris on severity[1] (%, bottom/middle/top of canopy) of mycosphaerella blight and seed yield of field pea at sites in Saskatchewan and Alberta in 1999.

Fungicide, Timing, Rate/ha			Mundare	Westlock	Star City SK		Prince Albert SK	
Fungicide	Early flower	Mid flower	Yield Mg/ha	Yield Mg/ha	Severity Aug. 4	Yield Mg/ha	Severity Aug. 23	Yield Mg/ha
1. Control			3.10 b	4.42 a	99/57/18	4.55 ab	100/64/20	4.16 c
2. Bravo	1.0 kg		3.13 b	4.44 a	85/42/7	4.66 ab	97/67/21	4.28 c
3. Bravo		1.0 kg	3.46 ab	4.04 ab	81/23/2	4.84 ab	100/78/28	4.56 abc
4. Bravo	1.5 kg		3.26 ab	4.11 ab	86/47/6	4.69 ab	95/48/15	4.92 abc
5. Bravo	1.0	1.0	3.18 ab	4.32 ab	66/16/1	5.02 a	90/45/8	4.89 abc
6. Quadris	125 g		4.17 a	4.52 a	95/62/16	4.28 b	92/49/17	5.19 ab
7. Quadris		125 g	3.29 ab	4.03 ab	97/51/10	4.72 ab	95/53/18	5.19 ab
8. Quadris	175 g		3.32 ab	4.42 a	98/48/6	4.82 ab	92/47/15	4.96 abc
9. Quadris	125	125	3.30 b	4.35 ab	87/41/9	4.71 ab	74/32/1	4.96 abc
10. Bravo Ultrex	1.0 kg		3.76 ab	3.74 b	97/64/12	4.77 ab	90/64/25	4.42 bc
11. Bravo + Quadris	1.0	125	2.91 b	4.25 ab	85/35/6	4.60 ab	80/34/10	5.19 ab
12. Quadris + Bravo	125	1.0	3.58 ab	4.51 a	85/24/3	5.02 a	85/37/3	5.31 a
$LSD_{0.05}$			1.00	0.68		0.61		0.88

[1] 3-4 weeks after the last fungicide application.

Table 9. Effect of Bravo, Dithane and Quadris applied during flowering on severity [1] of mycosphaerella blight on cv. Delta field pea (bottom/middle/upper canopy) and seed yield (bu/ac) at Prince Albert in 2000.

Fungicide, Timing, Rate/ha					
Fungicide	Early	Mid	Late	Severity[2] (%)	Yield (bu/ac)
1. Control				53/38/18	56 abc
2. Quadris	125 g			46/31/13	55 abcd
3 Quadris		125 g		44/27/10	59 ab
4 Quadris	125 g	125 g		41/27/12	58 ab
5 Quadris	125 g		125 g	44/29/9	56 abc
6 Quadris	175 g			42/26/11	45 d
7. Dithane	1.5 kg			46/28/14	50 bcd
8. Dithane	1.5 kg	1.5 kg		38/22/8	47 cd
9. Bravo Ultrex	1.0 kg			48/33/14	52 bcd
10.Bravo Ultrex	1.0 kg	1.0 kg		38/20/6	55 abcd
11. Bravo 500	1.0 kg			48/35/16	52 bcd
12. Quadris + Ultrex	125 g	1.0 kg		41/24/9	57 abc
13 Ultrex + Quadris	1.0 kg	125 g		39/24/8	57 abc
14. Bravo 500	1.0 kg	1.0 kg		36/23/5	63 a

[1] Percent leaf area affected by mycosphaerella blight at the bottom, middle, and top 1/3 of the canopy on August 14.

Decision support system - Pea

Results and Discussion: A decision support system (DSS) for field pea was developed, which was similar to the lentil system described previously. It consisted of a checklist of four categories: A) plant stand; B) number of days of rain within the past 14 days; C) the 5-day weather forecast and D) blight severity in the lower, middle and top 1/3 of the canopy. A total of 23 trials were used to assess the DSS. Our results indicate that cv. Carneval, which was selected for inclusion in many of these trials because it is one of the predominant cultivars grown in western Canada, did not respond to fungicide application in a consistent manner. Although it developed severe symptoms of mycosphaerella blight when disease pressure was high, the impact of blight on yield was much lower than for other cultivars. This indicated that Carneval is tolerant to this disease. In contrast, other recent studies report substantial yield increases from fungicide application. These studies have lead to the registration of several fungicides on field pea. In the current study, cultivars such as Alfetta (yellow pea) and Keoma (green pea) were susceptible, and often (but not always) showed a substantial yield increase from fungicide treatment when disease levels were high. However, foliar fungicide application for control of mycosphaerella blight was only rarely cost-effective, and does not appear to be an effective option for commercial producers in most years.

The combination of low commodity prices and inconsistent yield response from disease management worked against the DDS, and we concluded that it was not an effective tool for use by growers. As a result, the draft version of the DDS has been pulled from the website, and was never promoted and publicized as for the lentil DSS. The field pea DDS may be appropriate for many of the pea cultivars currently available, but more work is needed to determine which cultivars respond to fungicide treatment and which are tolerant to mycosphaerella blight. Despite the lack of success for the DDS, we feel that there were substantial benefits from this study. First, growers are now aware that it is rarely cost-effective to apply foliar fungicide to field pea (especially cv. Carneval). Second, if these apparent differences in tolerance among cultivars are confirmed, it will represent an important opportunity for improving disease management and increasing yield stability. For decades, breeders have screened pea collections for resistance, with little success. Shifting the focus from resistance to tolerance could open an important new avenue for improvement of pea cultivars in the prairie region.

The final assessments of each site using the DDS are still being summarized, and a scientific paper describing these studies is in preparation (Buchwaldt et al.).

Objective 2. MANAGEMENT OF ASCOCHYTA BLIGHT IN CHICKPEA

Background: Chickpea is a high-value crop that is adapted to the drier areas of the Canadian prairies. Its drought-tolerance and upright growth habit make it suitable even in rolling or slightly rocky sites. When this study was initiated, chickpea acreage was starting to increase, but it was still a very minor crop in Canada. In Saskatchewan, where most chickpea is produced, seeded area increased from 300 ha in 1995 to 350,000 ha in 2000. As a result, chickpea has become an important component of crop diversification in the southern prairie region, and much more effort was put into the chickpea portions of this work than had originally been planned. Studies of chickpea disease management were critical because diseases represent the most important constraint to its production, with ascochyta blight caused by *Ascochyta rabiei* leading the list as the most important disease of chickpea in this region. Partially resistant cultivars that are fairly well adapted to the prairies have become available in recent years, but it rapidly became apparent during the course of this study that they represented only part of the solution. The rapid increase of chickpea acreage in Saskatchewan in recent years, and the importance of ascochyta blight management to the success of this crop, made addition emphasis on studies of the biology and epidemiology of diseases on this crop of critical importance. Therefore, a range of studies were undertaken, in addition to those that were identified in the initial project proposal.

Method: In 1997, the efficacy of fungicides for management of ascochyta blight of chickpea was evaluated in two trials at the Saskatoon Research Centre farm at Saskatoon, SK. Each trial consisted of six replicates in a randomized complete block design. Each plot consisted of 8 rows, 3 m in length, with 0.3 m between rows. Four rows of barley were planted between plots to reduce interplot interference. One trial was sown to cv. Sanford, a partially resistant kabuli-type chickpea; the other to cv. Arizonia, a susceptible desi chickpea. The trials were seeded adjacent to one another; Sanford was seeded on 23 May and Arizonia on 02 June. Differences in seeding date were imposed to synchronize the flowering and subsequent development of the two cultivars. The plots were inoculated on 03 July 1997 with infected chickpea residue collected from a site with severe ascochyta blight on chickpea the previous year. There were 12 treatments in each trial; two rates of Bravo (1.0 and 1.5 kg a.i. ha^{-1}) applied one, two or three times, two rates of Quadris (125 and 250 g a.i. ha^{-1}) applied one or two times, an untreated control; and a positive control, where Bravo at 1.0 kg a.i. ha^{-1} was applied at 10-14 day intervals from the first treatment date until mid-September. Fungicides were applied on 16 July (early bloom), 23 July (mid-bloom) and 01 August (late bloom). Severity ratings were made on 18 July, 07 and 25 August. Desiccant (Reglone Pro) was applied on 25 September and the plots were harvested on 2-4 October. The general linear models procedure (SAS) was used to analyse the data.

In 1998, five trials were conducted in Saskatchewan at research farms in Saskatoon, Swift Current and Indian Head, and in commercial chickpea fields at Elrose and Sovereign. The partially resistant cultivars Sanford, Myles and B90 and the susceptible cv. Arizona were planted. The treatments included: unsprayed control, 1.0 kg a.i. ha^{-1} Bravo applied once (early flowering), twice (early and mid-flowering) and three times (early, mid- and late-flowering); Quadris at 125 and 175 g a.i./ha once (early flowering) and twice (early and mid-flowering). Ascochyta blight severity was rated two weeks after spraying using the Horsfall-Barrett scale (0-11).

In 1999, trials were established in commercial fields of chickpea cv. Myles at Harris SK (seeded May 4, 1999) and cv. Sanford at Zealandia (May 3). In 2000, trials were established in commercial fields of chickpea cv. Myles at Bladworth SK and cv. Sanford at Zealandia SK. In

both years, there were 12 fungicide treatments with the same experimental design, management and data analysis as described previously.

In addition, fungicide trials on chickpea were established at 6 of 7 AFIF spoke sites in 1999 and 2000. The treatments were generally similar to those reported here, and they were managed as part of this project, but the results have already been reported as part of an AFIF spoke project.

Results and Discussion: In 1997, fungicide application reduced blight severity late in the season (Table 10). In the partially resistant cv. Sanford, this reduction was small and did not translate into measurable yield increases. In the susceptible cv. Arizonia, the impact on blight severity was substantial, and this impact was reflected in subsequent seed yield. In Arizonia, the disease affected more than 70% of the leaf area in the controls during pod filling each year. The blight epidemic started early and developed slowly, but continued over a long period. As a result, more than one fungicide application was required to maintain disease control until harvest. There were no treatment effects in the earliest disease rating (mean 3% of leaf area affected in Sanford, 35% in Arizonia), so these data are not presented. Applications of Bravo and Quadris were equally effective in reducing the severity of ascochyta blight. A single application did not increase seed yield, but two (or more) applications increased yield by as much as 65% (Quadris at 250 gai). In Sanford, blight control with fungicide was not required to maintain yield.

In 1998, blight severity in the controls was 8-9% in cv. Sanford, 19% in cv. Myles, and 57% in the highly susceptible cv. Arizona (Table 11). Both Bravo and Quadris reduced ascochyta severity in cv. Sanford at Indian Head and Swift Current. Yield was low at Swift Current due to hot, dry conditions and fungicide treatments had no effect. Yields were higher at Indian Head and in the commercial fields at Elrose and Sovereign. Late applications of fungicide generally increased seed yield in the partially resistant cultivars, while an early application had no effect (Table 12). Despite the high levels of disease in the susceptible cv. Arizona at Saskatoon, fungicide application did not increase yield, probably because of variability of plant establishment in this particular trial (data not shown).

In 1999, blight severity was low (1-4%) at the first date of fungicide application in both sites. At the second application, levels had increased in controls to 14% in Zealandia and 72% in Harris (data not shown). Four weeks after the last application, ascochyta blight severity was 86% in Zealandia and 93% in Harris (Table 13). Most single applications did not increase yield relative to the control, except for Quadris applied at mid flower in Zealandia (Table 13). Treatments with two fungicide applications were required to increase yield, probably because of the early onset and rapid development of the disease epidemic. There was a trend for Bravo followed by Quadris to result in higher yields than Quadris followed by Bravo. A single early application of Bravo 500 or Bravo Ultrex at 1.0 or 1.5 kg a.i. was not enough to prevent yield loss. In Zealandia, a single application of a high rate (175 g a.i.) of Quadris at early flower reduced yield compared to the low rate (125 g a.i.).

In 2000, disease pressure was high in both commercial crops (Table 14). Ascochyta blight severity in the unsprayed plots was 1-5% at early flower and 5-25% at mid flower (data not shown), but increased to 80-90% later in the season. Seed yield was very low in unsprayed plots. Two applications of Bravo or Quadris, or an application of Bravo followed by Quadris, increased yield significantly. Increases in the rate of Bravo from 1.0 kg to 1.5 kg, or Quadris from 125 g to 175 g, did not affect efficacy. Dithane was less effective than Bravo or Quadris (Table14). An emergency registration for Bravo Ultrex was issued for Saskatchewan in 2000 because of a

shortage of Bravo 500. Our results indicates that there probably is no difference between these two formulations. Quadris is not yet registered for use in chickpea. However, data shows that Quadris has a slightly better effect on yield than Bravo. Quadris also received emergency registration in 2000 and 2001. Preliminary analysis of seed quality and seed-borne ascochyta infection show an positive effect of foliar fungicide application, (data not shown). Further research is also required to determine disease thresholds in relation to cultivar susceptibility, crop rotation, rainfall frequency etc.

An example of the date collected from the spoke sites is included in Table 15. Moderate levels of ascochyta blight developed at the Outlook and Scott sites. Under moderated disease pressure at these two locations, one and two application of Quadris increased yield significantly. Based on these results, it may be possible to extend the interval between two Quadris applications to about 20 days under moderate disease pressure.

Conclusion: In susceptible chickpea lines, ascochyta blight causes devastating losses in plant stand and yield. Where disease pressure is severe, multiple applications of fungicide are required to maintain crop yield, and even a regular application program may not be enough to save the crop. As a result of the high risks associated with ascochyta epidemics, very few growers are still producing highly susceptible cultivars. However, even the best cultivars are only partially-resistant. These trials supports the conclusions drawn from our studies on changes in disease reaction with plant age (Chongo, G. and Gossen, B.D. 2001. Plant age affects resistance to *Ascochyta rabiei* in chickpea. Can. J. Plant Pathol. xx: xxx-xxx. (In press)), which indicated that resistance declined as the chickpea plants mature. Due to this decline in resistance, fungicide application later in the flowering period often resulted in substantially increased seed yield in resistant lines. Fungicide application was cost-effective on these resistant lines because of the high value of the crop and the large potential for loss due to disease. However, all of our extension effort has emphasized that foliar fungicides are only one element of a disease management package that includes crop rotation, use of clean seed, resistant cultivars, sanitation and timeliness of operations to minimize the impact of disease.

We recommend that growers wait to apply a fungicide until a few isolated ascochyta leaf symptoms appear, which will depend on crop stage and weather conditions. Application should be made as soon as disease levels start to increase, but well before stem-break symptoms become widespread. Unlike lentil, application at late flowering can often be highly effective for management of ascochyta blight on chickpea. These recommendations are available on the website, in factsheets (Gossen, B.D., et al. 2001. Ascochyta blight of chickpea. Research Letter 02-01, May 11, 2001. Saskatoon Res. Cen., AAFC, Saskatoon. SK; Pearse, P., et al. 2001. Farm Facts: Ascochyta blight of chickpea. Saskatchewan Agriculture and Food, Regina. SK. 4 pp.) and have been presented at numerous grower meetings throughout the province. In addition, a scientific paper describing the results of this work, together with a more formal set of guidelines for growers, is in preparation (G. Chongo et al.).

Based on these studies, Quadris, which represents a new and highly effective group of fungicides, received emergency registrations to control ascochyta blight of chickpea in Canada and the USA in 2000 and 2001. The company is proceeding with an application for full registration on this crop, and is also proceeding with the development of Bravo Ultrex, which is being introduced to address certain environmental concerns associated with the current formulation of Bravo (Bravo 500).

Table 10. Ascochyta blight severity (% leaf area affected) and seed yield (Mg ha^{-1}) of two chickpea cultivars in inoculated field trials at Saskatoon, SK in 1997.

		cv. Arizonia			cv. Sanford		
	Timing of	Severity (%)		Yield	Severity (%)		Yield
Fungicide & Rate (ai h^{-1})	application	07 Aug	25 Aug	(Mg/ha)	07 Aug	25 Aug	(Mg/ha)
1. Untreated control		45	73	0.8	4	8	1.8
2. Positive control (Bravo)	1, 2, 3+	30	22	1.0	4	5	1.5
3. Bravo, 1.0 kg	1	36	63	0.9	6	6	1.5
4. Bravo, 1.0 kg	1, 2	28	43	1.0	4	5	1.6
5. Bravo, 1.0 kg	1, 2, 3	32	43	1.1	4	4	1.4
6. Bravo, 1.5 kg	1	27	75	0.8	4	8	1.8
7. Bravo, 1.5 kg	1, 2	24	42	1.2	4	5	1.4
8. Bravo, 1.5 kg	1, 2, 3	23	32	1.1	4	6	1.7
9. Quadris, 125 g	1	33	68	0.9	4	4	1.3
10. Quadris, 125 g	1, 2	32	36	0.9	4	5	1.4
11. Quadris, 250 g	1	44	68	0.8	4	3	1.4
12. Quadris, 250 g	1, 2	28	26	1.3	4	4	1.5
Mean		**32**	**49**	**1.0**	**4**	**5**	**1.5**
$LSD_{0.05}$		ns	21	0.36	ns	ns	ns

Timing of application corresponds to: 1) early bloom, 2) mid, and 3) late bloom.

Table 11. Effect of foliar application of Bravo and Quadris on ascochyta blight severity (%) on chickpea at five sites in Saskatchewan in 1998.

Fungicide, rate (a.i. ha^{-1}) & timing of applications		Elrose	Indian Head	Swift Current	Sovereign	Saskatoon
Untreated		19	8 a	8 a	9	57
Bravo, 1.5 kg	1	19	4 b	7 ab	8	32
Bravo, 1.5 kg	2	14	4 b	5 b	11	45
Bravo, 1.5 kg	3	21	-	-	12	37
Quadris, 125 g	1	21	6 ab	5 b	9	37
Quadris, 125 g	2	13	5 ab	6 ab	9	31
Quadris, 175 g	1	12	-	6 ab	12	39
Quadris, 175 g	2	16	-	5 b	9	48
$LSD_{0.05}$		ns	3	3	ns	ns
Contrasts	**df**					
Untreated vs fungicide	1	ns	**	*	ns	ns
Untreated vs Bravo	1	ns	**	*	ns	ns
Untreated vs Quadris	1	ns	*	*	ns	ns
Bravo vs Quadris	1	ns	ns	ns	ns	ns

Fungicide application at; 1) early, 2) mid, and 3) late flowering.
cv Sanford at Indian Head, Swift Current and Sovereign; Myles at Elrose; Arizonia at Saskatoon.
*,** Significant at $P \leq 0.05$, 0.01, respectively. ns = not significant.

Table 12. Effect of foliar applications of Bravo and Quadris on seed yield (Mg ha^{-1}) of chickpea at five sites in Saskatchewan in 1998.

Fungicide, rate (a.i ha^{-1}) & timing of applications		Elrose	Indian Head	Swift Current	Sovereign	Saskatoon
1. Untreated		1.28 dc	3.25	0.67	1.25 b	0.67
2. Bravo, 1.5 kg.	1	1.15 d	3.47	0.65	1.29 b	0.86
3. Bravo, 1.5 kg	1, 2	1.67 a	3.67	0.52	1.38 ab	0.88
4. Bravo, 1.5 kg	1, 2, 3	1.41 abcd	-	-	1.37 ab	0.85
5. Quadris, 125 g a.i.	1	1.31 bcd	3.46	0.54	1.29 b	0.87
6. Quadris, 125 g	1, 2	1.52 abc	3.46	0.63	1.56 a	0.99
7. Quadris, 175 g	1	1.44 abcd	-	0.48	1.42 ab	0.97
8. Quadris, 175 g	1, 2	1.62 ab	-	0.63	1.35 ab	0.93
$LSD_{0.05}$		0.33	ns	ns	0.22	ns

Fungicide application at 1) early; 2) mid, and 3) late-flower.
cv Sanford at Indian Head, Swift Current and Sovereign; Myles at Elrose; Arizonia at Saskatoon.

Table 13. Effect of foliar application of Bravo, Bravo ULTREX and Quadris on ascochyta blight severity (%) and seed yield (Mg ha^{-1}) in chickpea in two commercial fields in 1999.

Fungicide (a.i. ha^{-1})	Timing †	Harris		Zealandia	
		Severity††	Yield	Severity	Yield
Nontreated control		93 a	0.0 45 c	86 a	0.25 d
Bravo, 1.0 kg	Early‡	77 bc	0.41 bc	48 b	0.43 d
Bravo, 1.0 kg	Late	84 ab	0.26 c	37 bc	0.48 d
Bravo, 1.5 kg	Early	67 cd	0.37 bc	32 bcd	0.42 d
Bravo, 1 kg	Early + Late	33 f	0.81 ab	7 de	0.90 bc
Quadris, 125 g	Early	77 bc	0.54 bc	29 bcde	0.42 d
Quadris, 125 g	Late	77 bc	0.39 bc	16 cde	1.00 abc
Quadris, 175 g	Early	84 ab	0.25 c	45 b	0.25 d
Quadris, 125 g	Early + Late	28 f	0.82 ab	4 e	1.19 ab
Bravo ULTREX 1.0 kg	Early	81 ab	0.56 bc	50 b	0.38 d
Bravo + Quadris	Early + Late	56 de	1.24 a	5 e	1.28 a
Quadris + Bravo	Early + Late	50 e	0.43 bc	6 de	0.85 c
$LSD_{0.05}$		13	0.52	27	0.30

† Fungicide application at early (July 6 in cv. Sanford and July 14 in cv. Myles) and late-flowering (July 19 in cv. Sanford and July 29 in cv. Myles).

‡ Means in a column followed with the same letter do not differ based on LSD at $P \leq 0.05$.

†† Third and final disease rating (August 19 in cv. Sanford and August 29 in cv. Myles).

Table 14. Effect of foliar fungicides on ascochyta blight severity (% leaf area affected at the final sampling date) and seed yield of chickpea in commercial fields in Saskatchewan in 2000.

Treatment		Bladworth, cv. Myles		Zealandia, cv Sanford	
Fungicide & rate (a.i. ha^{-1})	Timing	Severity (%)	Yield (Mg ha^{-1})	Severity (%)	Yield (Mg ha^{-1})
Untreated control		81 a	0.54 f	93 a	0.60 d
Bravo, 1.0 kg	1	56 b	0.99 cde	59 de	0.71 abcd
Bravo, 1.0 kg	1 + 2	12 e	1.57 a	21 g	0.90 abc
Quadris, 125 g	1	31 cde	1.05 bcd	61 dce	0.74 abcd
Quadris, 125 g	2	49 cde	0.94 cde	45 ef	0.65 bcd
Quadris, 125 g	1 + 2	15 e	1.54 a	19 g	0.92 ab
Quadris, 175 g	1	31 cde	1.22 bc	44 ef	0.71 abcd
Bravo + Quadris, 1.0 + 125	1 + 2	29 de	1.34 ab	36 fg	0.94 a
Quadris + Bravo, 125 + 1.0	1 + 2	15 e	1.34 ab	34 fg	0.73 abcd
Bravo Ultrex, 1.0 kg	1	55 bc	0.68 ef	81 abc	0.63 cd
Bravo Ultrex, 1.0 + 1.0	1 + 2	29 de	1.19 bc	45 ef	0.59 d
Dithane, 1.7 kg	1	56 b	0.68 f	86 ab	0.62 d
Dithane, 1.7+1.7	1 + 2	67 ab	0.84 ef	70 bcd	0.54 d
$LSD_{0.05}$		24	0.32	21	0.28

† Fungicide timing; 1 = early, 2 = mid-flowering. ‡ Third and final disease rating.
Means in a column followed with the same letter do not differ based on LSD at $P \leq 0.05$.

Table 15. Foliar fungicide application reduces ascochyta blight severity (% leaf area affected at the last rating date = %S) and increases seed yield (Mg ha^{-1}) of chickpea at sites in 2000.

Treatment		Canora		Indian Head		Outlook		Scott		Swift Current	
Fungicide & rate (ai ha^{-1})	Timing	%S	Yield	%S	Yield	%S	Yield	%S	Yield	%S	Yield
Untreated control		19	1.14	5	0.72	45	0.71	21	0.38	5	2.38
Quadris, 125 g	1	12	1.22	4	0.66	7	1.16	14	1.19	5	2.51
Quadris, 125 g	2	12	0.79	3	0.61	8	1.69	14	1.09	4	2.58
Quadris, 125 g	3	12	1.25	4	1.24	8	1.37	23	1.02	4	2.49
Quadris, 125 g	1 + 3	14	1.07	3	1.19	7	2.2	16	2.11	3	2.58
Quadris, 175 g	1	14	1.06	4	0.79	11	1.13	14	1.46	4	2.54
Quadris, 175 g	2	12	1.1	4	0.8	28	0.87	26	1.5	4	2.54
Bravo Ultrex, 1.0 kg	1	16	0.86	4	0.91	8	1.57	23	0.74	3	2.68
Dithane, 1.7 kg	2	16	1.04	4	0.84	28	0.83	19	0.44	3	2.51
Mean		14	1.06	4	0.86	17	1.28	19	1.1	4	2.53
$LSD_{0.05}$		ns	ns	ns	ns	2.1	0.57	ns	0.5	ns	ns

† Fungicide applied at 1 = early, 2) = mid, 3 = late flower.

Means in a column followed with the same letter do not differ based on LSD at $P \leq 0.05$.

Sexual stage of ascochyta blight in Saskatchewan

Background: Ascochyta blight spreads rapidly on the prairies, and epidemics are sometimes observed in new production areas, even where apparently clean seed has been used. Similar observations have been made in the northwestern USA, where ascospores (produced by the sexual stage of the pathogen) are an important source of primary inoculum. These ascospores can be carried for long distances (miles) on the wind, so they contribute to long-distance dispersal of the pathogen. Asexual spores (conidia) generally move only a few metres within a field. Also, sexual recombination increases the risk of new races of *A. rabiei* developing, which could result in the breakdown of resistance in adapted cultivars. Two mating types (MAT-1 and MAT-2) are necessary for formation of the sexual stage of *A. rabiei*. Knowledge of the frequency and distribution of each mating type across the main chickpea growing region will help to determine the likelihood that the sexual stage (and its air-borne spores) will develop if conducive weather conditions occur. The objectives of this study were to assess the frequency of the two mating types required for sexual reproduction in this fungus, and to determine if the sexual stage is produced in the region.

Method: Tester isolates of know mating type were obtained from colleagues in the USDA. Stem pieces (4-5 cm) of 6- to 8-wk-old plants of cv UC 27 were harvested and oven-dried. The stems were surface sterilized in 1% NaOCl for 5 min, then placed in test tubes containing a mixed spore suspension of a tester isolate and the sample isolate. After 15 min, the stems were transferred to moistened sterile filter paper in a petri dishes and placed in the dark at 10°C for 7 wk. Each stem was then attached to the inner surface of a petri dish lid and inverted over water agar. If ascospores were discharge onto the agar, the sexual stage had formed. If the sexual stage formed with the MAT-1 tester, the sample isolate was MAT-2, and visa versa.

Samples of heavily-infected chickpea stubble were collected from one site in 1997 and 1998, and from all of the major chickpea producing areas of Saskatchewan and Alberta in spring of 1999 and 2000. The samples were examined for the presence of the sexual stage using the spore shower method (described above) and/or a wind tunnel for spore release.

Results and Discussion: The sexual stage was successfully produce in the laboratory. Of 44 isolates tested, 17 were MAT-1, 17 were MAT-2 and 10 isolates did not produce the sexual stage (Table 16). Both mating types were found at sites across Saskatchewan, often within the same field. The teleomorph (sexual stage) was not found in samples from 1997 and 1998, and from only one site in 2000. In 1999, asci were found in samples from seven sites, and mature ascospores were found at five of these sites. This is the first time that the sexual stage has been reported in Canada. These studies showed that the teleomorph (+ air-borne ascospores) is produced across the chickpea production areas of Saskatchewan in years with cool, wet springs, and may occasionally be a factor in spread of primary inoculum even in a cool, dry spring. The results of this work have been reported in Armstrong, C.L., Chongo, G., Gossen, B.D., and Duczek, L.J. 2001. Mating type distribution and incidence of the teleomorph of *Ascochyta rabiei* (*Didymella rabiei*) in Canada. Can. J. Plant Pathol. 23: 110-113, and a reprint of this paper is attached as Appendix 2.

In a companion study, (published in Armstrong, C.L. 2001. Ascospore production and

infection of chickpea by *Ascochyta rabiei*. M.Sc. Thesis, University of Saskatchewan, Saskatoon, SK.), exposure to freezing temperature at various stages in development did not appreciably affect the growth and maturation of the sexual stage, so growth could be initiated in the fall and then completed in the spring.

Table 16. Mating type of *A. rabiei* isolates collected from sites in Saskatchewan 1997-1999.

Location	Mat 1	Mat 2	Unknown
Saskatoon	4	8	2
Outlook, Zealandia, Plato			3
Elrose	4	2	1
Swift Current	1		
Eston	1	1	1
Kyle	1		
Sovereign	1	1	1
Vanscoy	1	1	
Oxbow		2	
Eston	1		
Vanscoy	2		1
Simmie		2	1
Rosetown	1		
Total	17	17	10

Conditions for infection by *A. rabiei*

Background: These studies were conducted to determine the optimal conditions for infection by *A. rabiei*. This information was required to provide consistently high levels of infection in studies aimed at characterizing the races of the pathogen present in western Canada (and abroad) based on differential infection on chickpea lines that carry different sources of resistance.

Method: In a series of growth cabinet studies, 3-wk-old plants of chickpea cvs. Sanford, Myles, B-90 (partially resistant) and UC 27 (susceptible) were inoculated with isolate Jan9702 of *A. rabiei* and incubated under humid conditions. The latent period (length of time from inoculation to the appearance of symptom) was recorded and disease severity was rated 2 wk after inoculation (Horsfall-Barrett scale). Where error variances were homogenous, repetitions of experiments were combined for subsequent analyses using analyses of variance

One study examined the impact of spore concentration (1, 2, 3, 4 and 5 x 10^5 spores mL^{-1}) on infection. Another study examined the impact of wetness duration on infection success; inoculated seedlings were incubated in a humidity chamber at 100% RH for 0, 12, 24, 36, 48, 60 and 72 h. In a growth stage study, plants of different ages (2, 4, 6, and 8-wk-old) were inoculated. Each study was repeated at least once.

A plant age study was also conducted in the field in 1999 and 2000 by planting the four cultivars on four seeding dates, with 2-wk intervals between dates. Two weeks after the final seeding date, the plots were inoculated with a spore suspension (2 x 10^5 spores mL^{-1}) of an aggressive isolate. Disease severity was rated 2, 4 , 6 and 8 wk after inoculation.

Results and Discussion: Increasing spore concentration from 1 to 5 x 10^5 conidia mL^{-1} resulted in increased disease severity, from 46 to 92% in UC27 and from 2 to 12% in Sanford. Disease severity increased with longer wetness duration, from 5 to 88% in UC27 and from 1 to 7% in Sanford (Table 17). Based on these studies, the inoculation protocol for characterisation of pathotypes of *A. rabiei* was set as follows: inoculation of 3- to 4-wk-old plants with 2 x 10^5 conidia mL^{-1}, followed by 48 h of incubation at 100% RH.

A paper describing changes in the disease reaction of resistant cultivars at various growth stages in the controlled environment and field studies is in press Chongo, G. and Gossen, B.D. 2001. Plant age affects resistance to *Ascochyta rabiei* in chickpea. Can. J. Plant Pathol.), and a draft of the text is presented in Appendix 3. In summary, UC 27 was highly susceptible at all plant ages (77-88% mean of leaves and stems). Sanford, Myles and B-90 were resistant at 2- and 4-wk after planting (3-19%), but susceptibility increased to 30-69% in plants that were at least 6-wk-old. The latent period on UC 27 was 4-6 days at all plant ages. On Sanford, the latent period was 10-11 days on 2-wk-old plants, and 7-8 days on 4- to 8-wk-old plants. Based on these observations, fields of these partially resistant chickpea cultivars will be highly resistant to ascochyta blight, even under high inoculum pressure and conducive weather conditions, until at least 4 weeks after seeding. However, epidemics may develop later in the growing season. In some years, fungicide application during flowering may be required to complement this resistance.

Table 17. Effect of spore density and leaf wetness period (controlled conditions) on blight severity (%) in two chickpea cvs inoculated with *Ascochyta rabiei* 2 wk after seeding.

	Spore density (x 10^5 conidia mL^{-1})					
Cultivar	1	2	3	4	5	$LSD_{0.05}$
UC 27	46	76	80	86	92	
Sanford	2	4	3	8	12	6

	Wetness period (h)						
	12	24	36	48	60	72	
UC 27	5	26	73	79	79	12	
Sanford	1	2	3	3	5	7	6

Impact of exudate on infection by conidia

Background: A unique feature of chickpea is that the plants secrete a highly acidic fluid from glandular trichomes on leaves, stems and pods. These secretions consist mainly of malate (approx. 60%) and oxalate (approx. 30%). Chickpea cultivars with higher levels of oxalic acid in their secretions are resistant to pod borer (*Heliothis* sp.) attack, but there is no clear association with disease reaction to ascochyta blight. Conidial germination is promoted by low concentrations of malic acid, but is inhibited as concentration increases. Germination of conidia on older plants is inhibited, which is unexpected because resistance of chickpea to ascochyta blight declines as plants reach flowering and podding. Despite increases in the volume and acidity of chickpea secretions as plants age, therefore, secretions fail to protect plants from infection as genetic resistance declines. Previous research suggests that secretions on young plants, or secretions on older plants diluted by dew or rain, could promote conidial germination and infection. In order to investigate this possibility, conidial germination was observed in serial dilutions of chickpea secretions. The effect of secretions on infection at two growth stages was ascertained by inoculating washed and unwashed plants. Simulated rainfall was used to determine if small volumes of water could remove secretions and affect infection by conidia of *A. rabiei*.

Materials and Methods:
Conidial Germination in Secretion Solutions: Preliminary trials were conducted to assess existing techniques for collecting exudates, and then to develop a novel technique (described below) to use for the study. Plants of two partially disease-resistant cultivars, CDC Marengo and Myles, were grown for 6 to 9 wk under in a greenhouse. Glandular hair secretions were collected by rinsing and/or dipping 103 whole plants in 1L of distilled water. The secretion solution was strained twice through cheesecloth and once through cheesecloth and rayon polyester cloth. The solution was freeze-dried over a 64 hr period using a lyophilizer. The dried secretion was resolubilized in 27 mL of distilled water and freeze-dried again to obtain 1.3 g of dried secretion, which was stored at 4°C until needed. A stock solution of 30mg mL^{-1} of freeze-dried chickpea secretion was used to prepare dilutions. This concentration is two times the seasonal average of malic acid concentration in chickpea secretions from one study, and about one-quarter the total organic acid content reported in another. The stock solution was serially diluted to give 0.024, 0.12, 0.6, and 3.0 mg mL^{-1} solutions.

A conidial suspension ($4x10^5$ conidia mL^{-1}) was prepared from cultures of *A. rabiei* isolate Jul 9804. Equal volumes of exudate solution and conidial suspension (20 µL each) were placed on well slides, bringing the conidial concentration to $2x10^5$ conidia mL^{-1} and the secretion concentrations to 0.012 , 0.06, 0.3 and 1.5 mg mL^{-1}. Distilled water was added to conidial suspension in the control treatment. Well slides were placed inside petri dishes containing moist filter paper and kept at room temperature. Germination of 100 conidia at 18 h and 200 conidia at 24 h was scored on each slide. Conidia were considered germinated when the germ tube length was equal to or greater than the width of the conidium. Each treatment was replicated three times, and the experiment was performed twice.

Osmotic potential and pH were measured for the 0.012, 0.06, 0.3, 1.5 and 30 mg mL^{-1}

exudate solutions. Measurements were also made for conidial suspensions (2 x and $4x10^5$). Analysis of variance using the general linear models procedure in SAS was performed on untransformed percent germination data. The two trials were compared by considering them as a split plot in time. Regression analysis was used to examine the relationship between germination and secretion concentration.

Effect on Infection at Two Growth Stages A conidial suspension was prepared as previously described. Chickpea cvs. Myles, Sanford, UC27 and CDC Marengo were chosen to represent partially-resistant and susceptible cultivars of both seed types. Plants were grown under greenhouse conditions. About 20 h prior to inoculation, all appropriate plants were washed with tap water to remove secretion buildup. The advance washing (Washed Prior) was intended to make experimental units more uniform and to reduce differences in plant wounding between treatments. Immediately before inoculation, plants in one treatment were washed again (Washed) to remove secretions. To remove moisture, plants were shaken lightly, set under fans in the growth chamber, and tapped gently with paper towel. When dry, plants in each replicate were inoculated with conidial suspension using a hand pump atomizer. Following inoculation, plants were incubated in a mist chamber composed of a plastic tent and humidifier. Disease severity was rated for each pot using the Horsfall-Barrett (0-11) scale 2 wk after inoculation. Disease severity data were analysed using the general linear models procedure in SAS. Due to heterogeneity of variance, data from trials within each experiment could not be pooled.

Whole Plant Trials Plants were inoculated 5 wk after seeding, in the late vegetative to early flowering stage. Plants at this growth stage have started to produce appreciable volumes of glandular secretions. Treatments in the first trial compared Washed and Washed Prior plants inoculated with two isolates of *A. rabiei* (Jul 9804 and Jan 9702). Inoculated plants were incubated in the mist chamber for 24 h. Due to poor emergence, plant number varied among pots. Each treatment was replicated three times in the randomized complete block design. In the second trial, an additional treatment consisted of plants that had never been washed (Never Washed) prior to inoculation. Only one isolate was used for inoculation (Jul 9804), after which plants were kept in the mist chamber for 48 h. Four replicates were included in this trial. Two similar trials were subsequently conducted using plants inoculated 10 wk after seeding, during the late flowering and early podding stage.

Rainfall Simulation Single plants of chickpea cultivars UC27 and CDC Marengo were grown in root trainers containing soil-less potting mix. A conidial suspension was prepared and plants were inoculated 5 wk after seeding. Plants were not washed the day before inoculation. To simulate rain indoors, an adjustable-spray garden nozzle was mounted on a 150 cm-high support and directed upwards. Plants were spaced 13 cm apart in two rows of 3 plants. Treatments received about 1, 2, or 4 mm of simulated rain in approx. 30, 60, or 120 sec. The volume of simulated rainfall given to each replicate was measured using a rain gauge. The 2-mm rain treatment was chosen to represent a typical volume of rain received in June in Saskatchewan. Other treatments are one-half and two times this typical volume. Following the simulated rain treatment, plants were shaken lightly and tapped gently with paper towel to remove excess moisture. When dry, the plants were sprayed with conidial suspension using a hand-pump

atomizer. Inoculated plants were incubated in a mist chamber consisting of a plastic tent and humidifier for 48 h. Each treatment was replicated four times in a randomized complete block design. Plants were rated using the Horsfall-Barrett (0-11) scale 2 wk after inoculation.

Results and Discussion:

Conidial Germination in Exudate Solutions In the preliminary trial, no conidia germinated in the 0.2%, 1% and 5% solutions of exudate collected in mineral oil. Collection of exudate by dipping plant material in mineral oil was very time consuming and required destruction of plant material.

Solutions of freeze-dried exudate had a significant effect on germination in all three tests. Results of germination counts at 18 and 24 hr were similar, so only the data from 24 hr will be presented. Results of each test could not be combined due to heterogeneous variance, so they will be discussed separately. In Test 1, germination in the control was very low (30%) (Fig. 1). Germination was promoted in the weakest exudate solutions, with germination increasing as the exudate concentration increased from 0.012 mg mL^{-1} to 0.06 mg mL^{-1}. Germination dropped to zero in the 0.3 mg mL^{-1} and 1.5 mg mL^{-1} solutions. In this test, there were significant linear and cubic relationships between germination and exudate concentrations.

Germination in the control in Test 2 was near 100%, and germination did not differ among the control, 0.012 mg mL^{-1}, and 0.06 mg mL^{-1} treatments. Germination was only partially inhibited in the 0.3 mg mL^{-1} solution, whereas no germination was observed in this treatment in Test 1. No germination occurred in the 1.5 mg mL^{-1} solution. There were significant linear and cubic components of the relationship between germination and exudate concentration in this test.

Test 3 followed a similar trend as Test 1. Germination was low (56%) in the control, and was promoted in solutions containing 0.012 mg mL^{-1} and 0.06 mg mL^{-1} of exudate. As in Test 2, germination of conidia was partially inhibited by the 0.03 mg mL^{-1} solution. Germination was completely stopped in the 1.5 mg mL^{-1} solution. In Test 3, the linear, quadratic, and cubic components of the relationship between germination and exudate concentration were significant.

In all three germination tests, conidial morphology was distorted in the 1.5 mg mL^{-1} and 0.3 mg mL^{-1} solutions. The distorted conidia were rounded, 10-12 μm in diameter, usually bearing a small protrusion at one end measuring 5-10 μm in length. Untreated conidia measured approx. 10 μm x 4 μm. Almost all conidia in the 1.5 mg mL^{-1} solution were distorted. In visual assessments of conidia in the 0.3 mg mL^{-1} solution, there was more variation in the degree of rounding of the conidia, and more of the protrusions measured about 10 μm in length than in the 1.5 mg mL^{-1} solution. Based on visual assessments, germ tubes formed in the 0.3 mg mL^{-1} solution tended to be wider and shorter than those in the control.

Viability of Conidia After Exposure to Exudate Solutions Conidia exposed to 1.5 mg mL^{-1} and 0.3 mg mL^{-1} chickpea exudate solutions for 24 hr showed the abnormal morphology (described previously). Dilution of 1.5 mg mL^{-1} and 0.3 mg mL^{-1} exudate solutions to 0.15 mg mL^{-1} and 0.03 mg mL^{-1} allowed conidia to germinate. After three hours in the diluted solutions, conidia still appeared rounded, but had germ tubes that were positioned opposite to the protrusions noted earlier. The germ tubes were quite short and broad in the 0.15 mg mL^{-1} solution, but were elongated and appeared normal in the 0.03 mg mL^{-1} solution.

By 20 hr following dilution, nearly all of the conidia in the controls had germinated (data not

shown). Conidia in the diluted exudate solutions had long germ tubes with relatively blunt ends. The rounded walls and small protrusions of the distorted conidia were still evident in both treatments. Occasionally, the original protrusion also produced a germ tube.

The effect of chickpea exudate on conidial germination is concentration dependent. Although there was some variation between the three runs of this trial, some general trends were apparent. In the two tests where germination of conidia in the control was low, dilute solutions (0.012 and 0.06 mg mL^{-1}) of exudate promoted conidial germination. This is consistent with results from some previous reports. Promotion of germination was not apparent in Test 2, since germination in the control was already at 91%. A 1.5 mg mL^{-1} solution of freeze-dried exudate completely inhibited conidial germination, and germination was partially or completely inhibited in 0.3 mg mL^{-1} solutions. Conidial germination occurred in solutions containing 0.06 mg mL^{-1} of exudate or less. Due to the wide variation in the reported organic acid concentration in exudate, it is difficult to determine how much dilution of exudate would be necessary to allow conidial germination on plant surfaces. If the organic acid content of exudate is low (ie. 0.27 mg mL^{-1}), dew might dilute the exudate sufficiently to allow germination of *A. rabiei* conidia and subsequent infection. However, if the organic acid content of chickpea exudate is closer to the top end of the reported range at 126 mg mL^{-1}, rainfall would likely be required to remove or dilute exudate before conidial germination could occur.

Conidia exposed to 1.5 mg mL^{-1} and 0.3 mg mL^{-1} chickpea exudate solutions for 24 hr were morphologically distorted, but remained viable. Dilution of these exudate solutions to 0.15 mg mL^{-1} and 0.03 mg mL^{-1} allowed conidia to germinate. Although germination after diluting the exudate appeared to progress normally, the physical distortions of the body of each conidium were still apparent.

Effect on Infection at Two Chickpea Growth Stages Disease severity on chickpea plants inoculated at 5 wk or 10 wk after seeding was significantly affected by washing treatment, chickpea cultivar, and their interaction. A third treatment was introduced into the second repetition of the 5-wk and 10-wk trials, so data from the repetitions within each trial were not pooled. The general pattern of results was similar between repetitions of the 5-wk and 10-wk trials. Only the data from Test 2 of each trial are presented.

In Test 1 on 5-wk-old seedlings, severity was 26-36% higher on plants of the susceptible cvs. CDC Marengo and UC27 in the Washed at Inoculation treatment than on plants in the Washed Prior treatment. Severity did not differ between plants of the partially-resistant cvs. Myles and Sanford in the Washed at Inoculation and the Washed Prior treatments (data not shown). In Test 2 on 5-wk-old seedlings, disease severity was higher (13 to 61%) on Washed at Inoculation than on Washed Prior plants of cvs. CDC Marengo, UC27 and Myles (Fig. 2). For the partially-resistant cultivar Sanford, there was no difference in disease severity between plants in the Washed at Inoculation treatment and plants in the Washed Prior treatment.

Differences between disease severity on Washed at Inoculation and Washed Prior Myles plants were only observed in Test 2. The higher infection level on Washed at Inoculation Myles plants in Test 2 than in Test 1 was likely due to a difference in incubation conditions. In Test 1, plants were incubated in the mist chamber for only 24 hr, whereas the incubation period in Test 2 was 48 hr. This longer wet period in Test 2 appears to have allowed infection of Myles plants in the Washed at Inoculation treatment, but exudate on plants in the Washed Prior treatment

inhibited infection.

Test 2 on 5-wk-old seedlings included an additional treatment consisting of plants that were not washed the day prior to inoculation (Never Washed). Disease severity on each cultivar was similar for plants that were Washed Prior and plants that were Never Washed (Fig. 2). The differences between these treatments for Myles, UC27 and CDC Marengo were very small (<5% severity), inconsistent, and probably not biologically meaningful.

For all four cultivars inoculated at 10 wk after seeding, disease severity was higher on plants in the Washed at Inoculation than Washed Prior treatment in both tests. The difference between the Washed at Inoculation and Washed Prior treatments ranged from 11 to 44% for the susceptible cvs. CDC Marengo and UC27 in Tests 1 and 2. Relatively small (3 to 16%) differences were observed between the Washed at Inoculation and Washed Prior treatments in the resistant cvs. Myles and Sanford in both tests. In Test 2 on 10-wk-old plants, disease severity on susceptible cultivars was 15 to 39% higher on plants in the Washed Prior treatment than Never Washed treatment. For partially-resistant cultivars, there was no difference in disease severity between plants in the Washed Prior and Never Washed treatments.

Inoculation of plants at 5 or 10 wk after seeding showed that a washing treatment prior to inoculation can remove exudate and increase infection of susceptible cultivars. The washing treatments, however, may have also removed plant nutrients, dust particles, and microorganisms from the plant surface that might also influence infection. For the resistant cvs. Myles and Sanford exudate had no effect on infection when inoculated at 5 wk after seeding, due to their strong genetic resistance at this growth stage. When inoculated at 10 wk, exudate slightly inhibited infection in the Washed Prior and Never Washed treatments.

In susceptible chickpea cultivars inoculated at 5 wk, there was no difference in disease severity between plants in the Washed Prior treatment and plants in the Never Washed treatment. Chickpea plants do not produce large volumes of exudate until the late vegetative stage, so there is a limited accumulation of exudate on plants at 5 wk after seeding. The amount of exudate on plants, therefore, would be similar on plants in the Never Washed and Washed Prior treatments. In contrast, plants inoculated at 10 wk have more time to accumulate exudate on their surfaces. Reduced levels of exudate on susceptible 10-wk-old plants in the Washed Prior treatment, therefore, might explain why these plants developed more severe blight infection than plants in the Never Washed treatment. No differences in disease severity were evident on 10-wk-old plants of the resistant cultivars between the Washed Prior and Never Washed treatment. Blight severity was low (2 to 22%) on the resistant cultivars inoculated at 10 wk, and any effect of exudate concentration on infection in these cultivars was too small to be measured.

Rainfall Simulation

The volume of simulated rainfall applied to plants prior to inoculation had a significant effect on disease severity in both tests. Disease severity did not differ between the two susceptible cvs. UC27 and CDC Marengo, so these data were pooled for further analysis. Data from both tests showed similar trends and their variances were homogeneous, so only the mean data are presented. Single degree of freedom contrasts indicate that there was a significant linear relationship between disease severity and rainfall volume in both tests. Regression analysis was used to describe the relationship using an equation, given by [$y = 22.7 + 13x$, $r^2 = 98.7$] where y = severity (%) and x = rainfall (mm). In other words, disease severity increased steadily as the

volume of rain increased between 0 and 4 mm.

The simulated rainfall trial indicates that infection of susceptible cultivars increases as rainfall volume increases from 0 to 4 mm. The 1, 2, and 4 mm rainfall treatments are reasonable volumes to expect under Saskatchewan conditions, although the intensity (2 mm min^{-1}) was very high. According to a model for predicting the risk of ascochyta blight outbreaks, daily rainfall volumes of 3.4 mm contribute to epidemic development, provided that such events are sufficiently frequent. In light of the rapid spread of ascochyta blight in chickpea under prairie conditions, it is evident that even relatively small rainfall events effectively disperse the pathogen and provide suitable conditions for infection.

Conclusion: This study provided interesting insights into the factors that affect disease progress of ascochyta blight on chickpea, but the impact of exudate is too small relative to the effects of genetic resistance or fungicides to have an impact on practical disease management. However, Cheryl Armstrong-Cho, the M.Sc student who conducted this work, intends to write it up as a paper for a scientific journal.

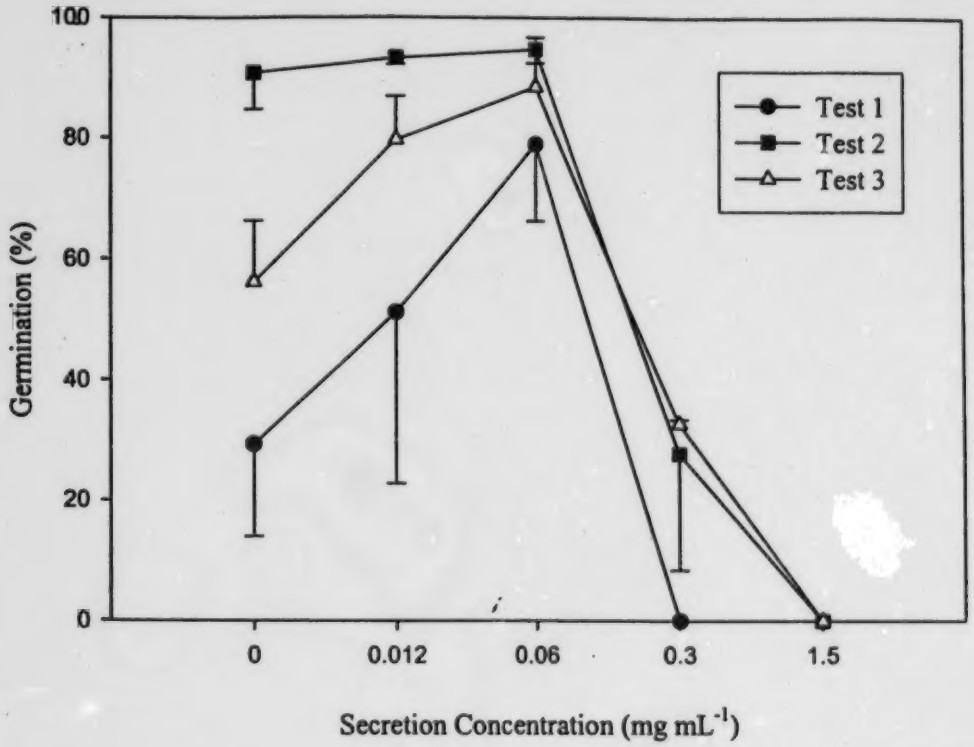


Figure 1 Germination of *Ascochyta rabiei* conidia after 24 hr in solutions of chickpea glandular hair exudate. Capped bars represent standard error.

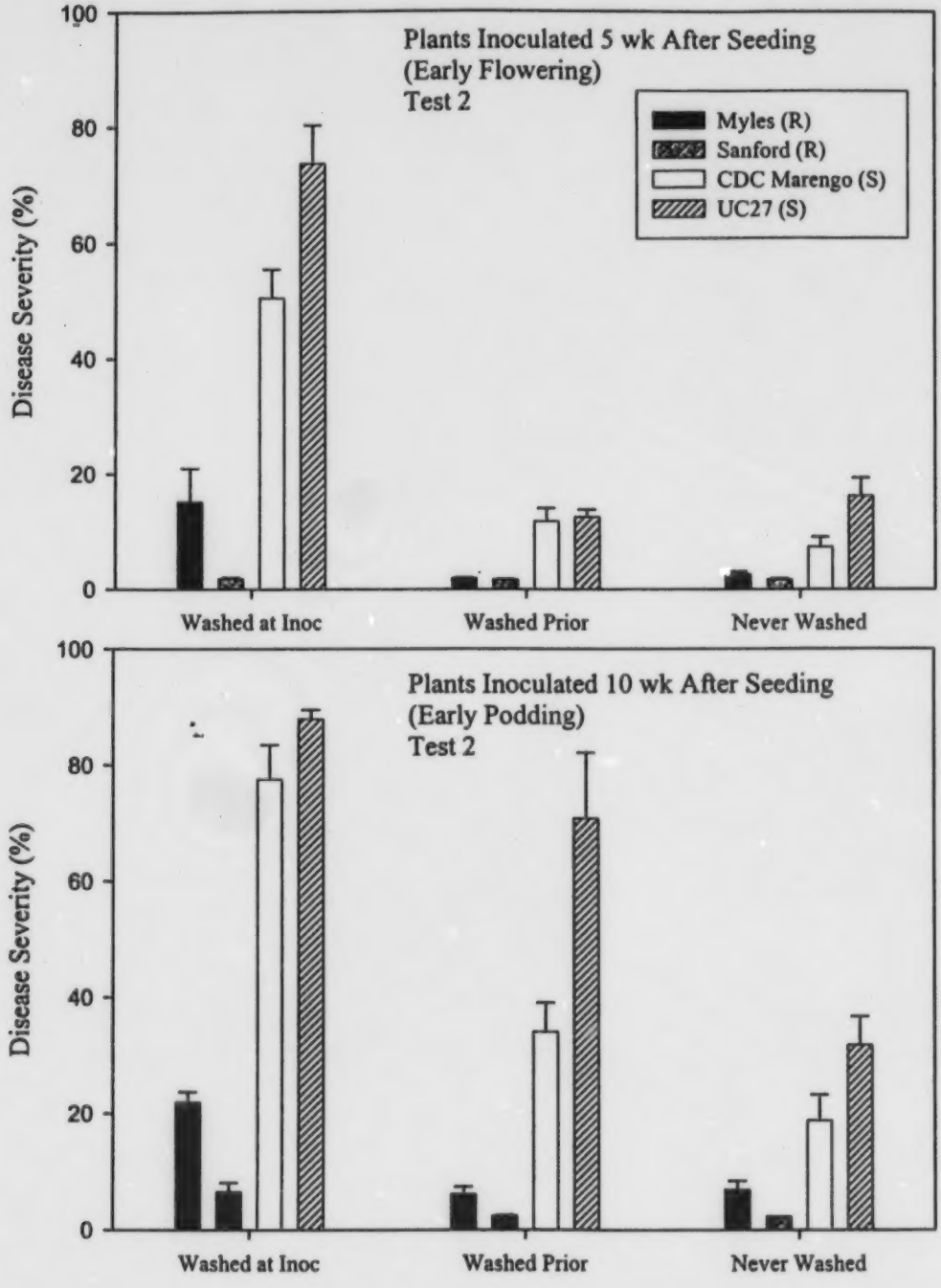


Figure 7. Ascochyta blight severity on chickpea plants with pre-treatments to produce different levels of glandular hair exudate (Washed at Inoc = rinsed immediately before inoculation, Washed Prior = rinsed 24 hr before inoculation, Never Washed = untreated control). R = partially blight-resistant cultivar, S = blight-susceptible cultivar. Capped bars represent standard error.

Survival of *Ascochyta rabiei* in Saskatchewan

Background: *Ascochyta rabiei* survives on infected chickpea debris. It is important to determine how long it survives, because of its effect on the impact of crop rotation and tillage practises on disease levels in subsequent crops. Many other studies have examined this question in regions where the weather conditions are very different from those on the Canadian prairies, but it has never been critically examined in a region with a continental climate.

Field study, Saskatoon

Method: A site on the AAFC Research Farm at Saskatoon with severe ascochyta blight in 1996 was used for this study. The area was planted to barley in 1997. In 1998-2000, portions of the test area were seeded to the susceptible chickpea cv. Cheston and the remainder to wheat (cv. AC Majestic). In 2001, every plot was seeded to chickpea and severity was rated (June 29, July 10 and July 27) in chickpea plots 1, 2 and 3 years after the most recent epidemic. Each year, when disease observations were completed, the crop was clipped and removed to minimize the movement of inoculum between plots. Therefore, no yield data was collected.

Results and Discussion: In 1998, eight chickpea plots were available which planted two years after the previous ascochyta epidemic. On July 16, ascochyta severity was 2.8-2.9 on the Horsfall-Barrett scale in all plots. Warmer and drier weather conditions than normal prevented a severe epidemic. In 1999 and 2000, cool, wet conditions pushed the epidemic; blight was severe in all plots. Many of the plants were killed before the crop residue could be removed.

In 2001, conditions were cool and dry in early spring, so chickpea germination was very slow. There was a record drought throughout the summer, so conditions for disease increase were very marginal. At the first rating date, mean ascochyta blight severity (based on unadjusted HB ratings) were 0.5, 0.9 and 4.3, respectively, for treatments seeded to chickpea in 1998, 1998 and 2000. On the second date, values were 0.9, 2.1 and 4.8. On the third date, they were 1.8, 2.9 and 3.9. Even under these extreme drought conditions, blight caused extensive damage in plots seeded on stubble from 2000, and was present at moderate levels in plots on chickpea stubble from 1999. Disease severity in the plots on chickpea stubble from 2000 were equal to that in the two continuous chickpea plots at the south end of the trial. Ascochyta blight was present at the first and second rating dates in all of the plots on chickpea stubble from 1998, but at lower levels than from 1999. The extremely dry conditions that occurred in the spring of 2001 makes it extremely unlikely that this resulted from infection by ascospores produced outside the plot area. We conclude that stubble-borne inoculum can survive for at least three years, but that the amount of inoculum declines steadily with time.

Field study, Swift Current

Method: A variety of different pulse crops were evaluated each year from 1994-1996 in a series of large pulse crop trials at the AAFC Research Centre Farm at Swift Current. Each of these trials was planted adjacent to the previous trial, and an epidemic of ascochyta blight was observed in plots of the susceptible chickpea cv. Cheston (desi type) each year. Each trial was a 4-replicate RCBD, with plot 2.4 x 6 m. Following the pulse trial, the study area was seeded (zero-till) to spring wheat each year. In 1997, the exact locations of the chickpea plots within

each trial were identified using GPS technology. In 1998, the entire study area was seeded to cv. Cheston. Blight severity was compared (HB ratings) at two dates to compare the initial disease severity in the chickpea crop at sites that have been out of chickpea for 1, 2 and 3 years.

Results and Discussion: Twelve chickpea plots were available, which were planted 2, 3 and 4 years after the previous ascochyta epidemic. On June 26, blight severity was 4.0 after one intervening crop, and 1.0 after two or three crops of cereals. On July 14, ascochyta severity was 7.0 after only one intervening crop, 2.0 after two crops and 1.0 after three crops. The results showed that inoculum survived on 2-yr-old chickpea stubble, but declined by the third year.

Residue burial

Method: This trial was designed to augment information from other field studies regarding changes in the survival of *A. rabiei* in the field over time. Chickpea residue that was heavily infected with *A. rabiei* was collected at Saskatoon in the fall of 1997 and air-dried. Chickpea stem pieces, and leaf/pod material of uniform weight, were enclosed in nylon-mesh bags and buried at 0-, 5- and 10-cm depth in a clay-loam soil at the AAFC Research Farm at Saskatoon on February 18, 1998, under a deep snow cover. Samples were retrieved in May and October of 1998, 1999, 2000 and in May 2001. There were six replicate bags per sampling date. One set of samples was assessed as a baseline at the start of the trial. After collection, ½ of each sample (by weight) was oven-dried and weighed to monitor the breakdown of chickpea debris over time. The remaining material was rinsed in running water for 30 min, then ½ was plated onto water agar plus streptomycin, incubated on a lab bench for 5-10 days, then the number of pycnidia were counted. The remaining 1/4 of the original sample was chopped in a blender in 40 mL of sterile water to release spores and fragments of mycelium, filtered through cheesecloth, and inoculated onto 14- to 21-day-old seedlings of the susceptible chickpea cv. UC 27.

Results and Discussion: In the plating study (Table 18), the pathogen population increased from the initial collection in 1997 to the spring of 1998. As in a previous study on survival of *A. lentis* on lentil residue, the number of pycnidia produced is generally higher in the spring than in the fall of the previous year. In May 1999, all of the samples were overgrown with *Fusarium* spp., so no assessments could be made. However, by the fall, there was little or no contamination with other species. In 2001, more than 4 years after the infested residue was collected, the pathogen was still present in the material, irrespective of depth of burial. The data was highly variable, but there was no clear pattern of difference among the depth-of-burial treatments.

In the bioassay (Table 19), the number of lesions produced from these samples was generally high in the original collections and declined slowly until the spring of 1999. By the fall of 1999, the number of infections declined sharply. The only exception to this pattern was stem lesions from stem material. The number of stem lesions produced was initially low, rose sharply in the spring of 1998 and then dropped to low levels over the following year.

These results support the results of the field trials, which indicate that at least 2 (and preferably 3) crops between successive chickpea crops is required to reduce pathogen populations in infested residue to acceptable levels. It is also important to note that pathogen populations in the residue are often higher in the year following a chickpea crop than they were at harvest the previous year, which is normally the height of the epidemic.

Table 18. Mean number of pycnidia of *Ascochyta rabiei* per sample in a plating test of infested chickpea leaf/pod or stem residue buried at 0-, 5- or 10-cm depth in a heavy clay soil at Saskatoon, SK, 1997-2001.

Sampling dates	# Pycnidia ('000s)					
	Leaves (1.9 g)			Stems (per 25)		
	0	5	10	0	5	10
Fall 1997	1.4	.	.	0.9	.	.
May 1998	10.2	21	4.2	50.6	21.6	24.7
Oct 1998	6.3	4.1	7.9	18.6	40.1	13.1
May 1999	nd	nd	nd	nd	nd	nd
Oct 1999	10.5	8.3	11.9	25.7	14.8	10.2
May 2000	22.1	16.2	12.8	34.4	22.9	28.3
Oct 2000	13.7	8.7	9.4	16.2	12.4	5.4
May 2001	6.9	8	9.2	51.6	35.4	22.1

Table 19. Mean number of leaf or stem lesions of *Ascochyta rabiei* per sample in a bioassay of infested chickpea leaf/pod or stem residue buried at 0-, 5- or 10-cm depth in a heavy clay soil at Saskatoon, SK, 1997-2001.

Sampling dates	# Leaf lesions						# Stem lesions					
	Leaves			Stems			Leaves			Stems		
	0	5	10	0	5	10	0	5	10	0	5	10
Fall 1997	109	109	109	31	31	31	59	59	59	8	8	8
May 1998	59	30	7	58	53	45	36	12	2	54	33	25
Oct 1998	33	31	51	59	34	34	4	5	4	11	7	8
May 1999	33	24	37	32	25	27	7	1	16	3	2	2
Oct 1999	15	12	14	21	20	24	1	0	1	1	3	2
May 2000	18	21	18	14	8	15	2	3	1	0	1	1
Oct 2000	9	10	8	12	11	14	0	0	0	0	0	0
May 2001	8	7	8	12	22	26	1	0	1	0	7	5

Objective 3. IDENTIFYING PATHOTYPES OF *A. RABIEI*

Background: Pathogenic variability in *A. rabiei* has been reported from many parts of world, including India, Pakistan, Syria, Lebanon, the USA and Italy. Breeding programs have used this information to develop resistance cultivars, but lines that are resistant in one region are often susceptible at other locations. Efforts have been made to separate the pathotypes involved in these differential reactions, but the literature is fragmented and incomplete. The objectives of this study were to assess pathogenic variability in a Canadian population of *A. rabiei* isolates using a differential set of chickpea lines/cultivars and to compare this variability with that of a group of foreign isolates. We need information on the pathotypes of *A. rabiei* that occur in western Canada in order to assess the potential for breakdown of resistance in existing chickpea lines, and to determine the best approach(es) for developing new sources of resistance.

Materials and Methods: A large group of blight-resistant chickpea lines/cultivars (29 desi lines from ICARDA, 20 kabuli lines from ICRISAT, and 11 lines from CDC, University of Saskatchewan), were assessed to select those that had a differential reaction among Canadian isolates of *A. rabiei*. The commercial kabuli cultivars UC27 (susceptible) and Sanford (resistant) were included as checks. In total, 62 chickpea lines were assessed using three aggressive isolates. From these, 14 lines were selected and used to screen a population of 40 Canadian isolates and 18 isolates from other countries.

The Canadian isolates were collected from 25 infected chickpea fields across Saskatchewan from 1997 to 2000. The foreign isolates consisted of five isolates from Australia, five from the USA, six from Syria, two from India and two from Turkey.

Due to space limitations, only 10 isolates could be screened on the 14 differential lines at one time. To screen a batch of 10 isolates, 10 pots of each line were seeded, with three seeds per pot, in a soil-less mix of peat moss, vermiculite and sand (2:2:1 v/v/v) plus slow-release granular fertilizer. The pots were placed in a greenhouse for 3 weeks. One pot of seedlings per line was then inoculated with a suspension of 2×10^5 conidia mL^{-1} of each isolate, and the inoculated seedlings were incubated in a misting chamber held a growth chamber at 20:16°C (day:night) temperature and 16 h photoperiod with 250 $\mu E^{-2} s^{-1}$ After 48 h, the plants were moved from the misting chamber to an adjacent bench in the same growth chamber and arranged in a completely randomized design. Seedling age, inoculum concentration and incubation period for the trial were selected based on a series of preliminary trials to optimize each factor.

Each plant was rated 14 days after inoculation using a 0-9 rating scale where: 0 = no symptoms; and 9 = plants dead. Inoculation of each batch of 10 isolates was repeated once. The disease reaction of the differentials was assessed using analysis of variance (SAS Institute, Carry, NC). Based on the interaction phenotype of each differential by isolate combination, four classes of the 0-9 rating scale was consolidated as follows: 0, 1 = resistant (R); 2, 3 = moderately resistant (MR); 4, 5 = moderately susceptible (MS), 6, 7 = susceptible (S), 8, 9 = highly susceptible (HS). For grouping isolates into pathotypes, the data was further consolidated into two categories: 0-3 = R and 4-9 = S.

Cluster analysis was used to examine relationships among the Canadian and foreign isolates (NTSYS-pc program, Exeter Software, USA). The isolate by chickpea line dataset was clustered using the unweighted pair group method, using arithmetic averages and average

distance. Standardized data showed no difference in cluster analysis, so the final clustering was performed on unadjusted means. The clustering method was tested for goodness-of-fit by computing a cophenetic value matrix, which was then compared to the original data.

Results and Discussion: During the initial screening of 62 chickpea lines/cultivars to select a differential set, several factors were considered. First, in a preliminary assessment of all the chickpea lines with three isolates, only lines that produced a consistent differential reactions were selected. Second, when several lines came from similar parental source material, only the one with the clearest and most consistent pattern of differential reaction was selected. Third, limited amounts of seed were available for the lines from ICARDA and ICRISAT, so seed multiplication under controlled conditions was required. Lines that readily set seed with good germination under these conditions were preferred. In total,14 lines were selected to screen the *A. rabiei* isolates, which was further reduced to only eight differential lines in the final data analyses.

In analysis of variance, the interaction of host and isolate was significant, both for the Canadian isolates only and in the combined analysis of Canadian and foreign isolates. This demonstrates that the reaction of some isolates was specific to certain differential lines. Based on these differential reactions, 15 pathotypes (races) were identified in the Canadian population (Table 20). These ranged from those that were only weakly pathogenic on the highly susceptible cv. UC27, to those that were virulent on as many as 6 of 8 differential lines. The high level of pathogenic variability among isolates was also shown by the presence of different pathotypes even within single fields (Table 21). In fields where two or more isolates were collected, we identified two or more pathotypes in 6 of the 7 fields. This supports the conclusions of several other studies conducted around the world, which have all reported substantial pathogenic variations in populations of *A. rabiei*.

Seedlings of cv. Sanford were susceptible to pathotypes 11, 12, 14 and 15, which represented 33% of the Canadian isolates in the study. Sanford is one of the very few chickpea cultivars that is adapted to the short growing season of the Canadian prairies and also carries a useful level of resistance to ascochyta blight. As a result, it is currently the most widely-grown chickpea cultivar in this region. These results suggest that its useful life as a resistant cultivar in Saskatchewan may be quite short. No isolates were pathogenic on line ILC 4421, 5% were pathogenic on Flip 48-83 and ILC 3856, and 20% on ICC 6328. Although line ILC 4421 was resistant to all of the isolates tested, it was reported as susceptible at other sites around the world. This difference in disease reaction among sites is almost certainly due to differences in virulence in local pathogen populations. Lines ILC 4421 and Flip 83-48 also represent potential sources of resistance for use in western Canada. The reaction of all three resistant lines needs to be assessed against a larger population of isolates and in the field before a major effort is made to incorporate this resistance into adapted chickpea lines.

Cluster analysis of the isolates showed a relatively high cophenetic correlation ($r = 0.83$), indicating a good fit of isolates within clusters. Three major clusters were identified (Fig. 3). Isolates in the first major cluster were weakly pathogenic or nonpathogenic and contained only pathotype 1. In the second major cluster, isolates were pathogenic on one or two differentials (pathotypes 9 and 12). In the third major cluster, which contained the rest of the pathotypes, all of the isolates were pathogenic. Their disease reaction on the differential lines was highly variable, resulting in many sub-clusters. Most of the foreign isolates were pathogenic, so they occurred in

the second and third clusters (Fig.3). Isolates representing two of the six races previously described from Syria (race 2 and 3) clustered together and were not pathogenic on these differentials. There was some grouping of isolates based on locality; 4 of 5 isolates from Australia clustered together, as did both isolates from Turkey, and 2 of 3 isolates from the USA. However, at least one Canadian isolate occurred in each sub-cluster that contained a foreign isolate. In general, pathogenic variability was noted among isolates from within and between countries.

This study demonstrated that there is a broad range of pathogenic variation in the population of *A. rabiei* in western Canada; 15 races were identified using eight differential chickpea lines. Only one line was resistant to all 40 Canadian isolates. Faced with a genetically diverse, sexually reproducing pathogen population, plant breeders need to assess the potential for developing cultivars with durable forms of resistance. Also, pathologists need to monitor changes in the race structure of the pathogen population to predict the breakdown of resistance in existing chickpea cultivars.

Table 20. Disease reaction of eight chickpea differential lines to 40 isolates of *Ascochyta rabiei*.

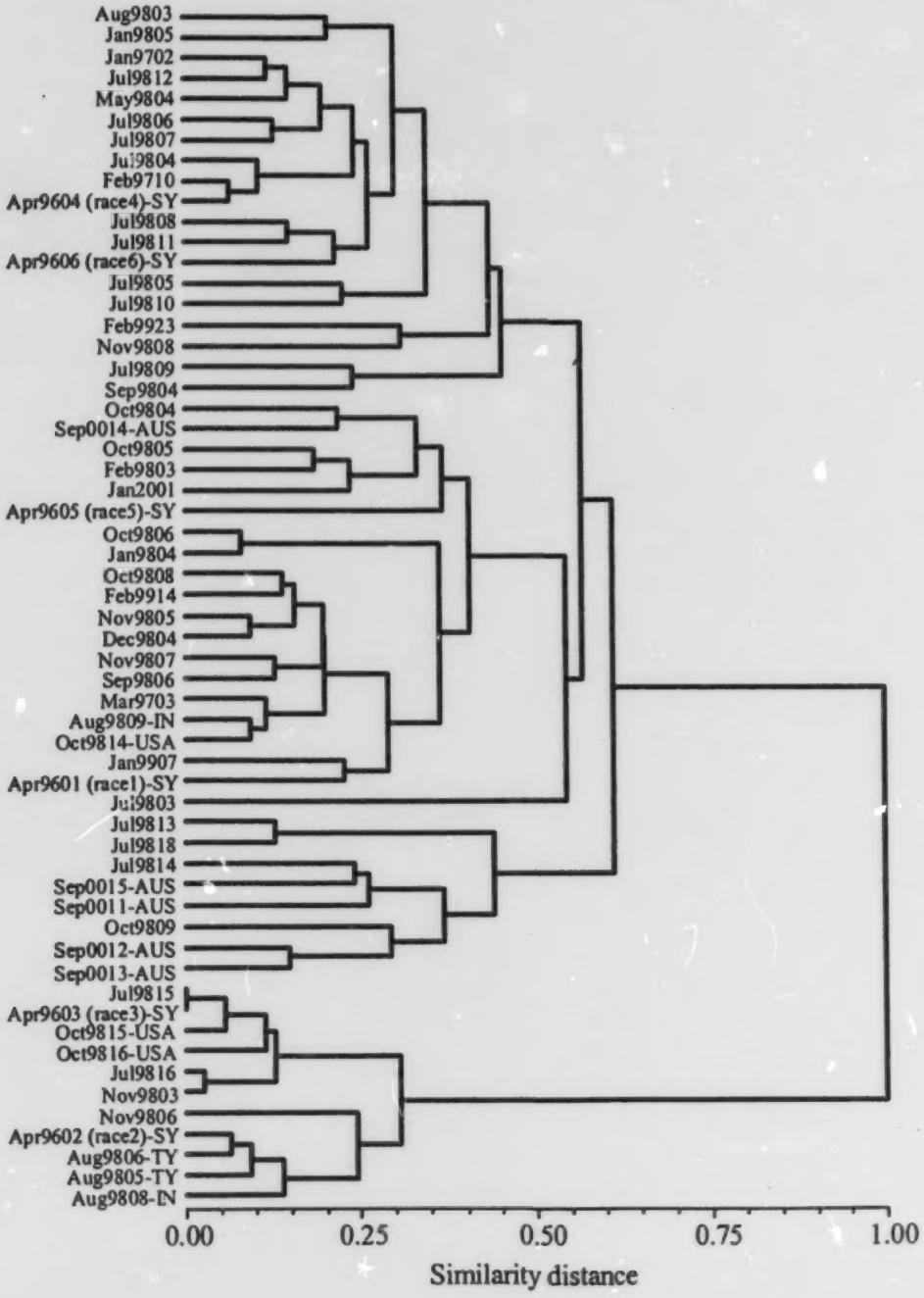
		Host differential							
Pathotype	Isolate	UC27	ICC4200	ICC4475	ICC6328	Sanford	ILC3856	FLIP 83-48	ILC442
1	Jul9815	MR	R	R	R	R	R	R	R
	Jul9816	MR	MR	MR	MR	R	R	R	R
	Nov9803	MR	MR	MR	R	R	R	R	R
	Nov9806	R	MR	R	R	R	R	R	R
2	Oct9808	HS	MR	MR	MR	MR	R	R	MR
	Nov9805	HS	MR	MR	MR	MR	MR	MR	R
	Dec9804	HS	R	MR	R	R	R	R	R
	Feb9914	HS	MR	MR	R	MR	R	R	R
	Jan9907	HS	R	R	R	R	R	R	R
	Mar9703	HS	MR	R	MR	R	R	R	R
	Nov9807	HS	MR	MR	MR	MR	R	R	R
	Sep9806	HS	MR	MR	MR	MR	R	R	R
3	Oct9805	HS	S	MR	MR	R	MR	R	R
	Feb9803	HS	MS	MR	R	MR	MR	R	R
	Jan2001	HS	MS	MR	MR	MR	MR	R	R
4	Aug9803	HS	S	S	MR	MR	MR	MR	MR
	Jan9702	HS	MS	MS	MR	MR	MR	MR	R
	Jul9812	HS	MS	S	MR	MR	MR	MR	MR
	Jul9806	HS	S	MS	MR	R	R	R	R
	Jul9807	HS	S	MS	MR	MR	R	MR	MR
5	Jul9808	HS	MS	MS	MS	MR	MR	R	MR
	Jul9810	HS	MS	S	MS	MR	MR	R	MR
	May9804	HS	MS	MS	MS	MR	MR	MR	R
6	Jan9805	HS	MS	S	MS	MS	MR	R	MR
	Jul9811	HS	MS	MS	MS	MS	MR	MR	MR
	Sep9804	HS	S	S	S	MS	MR	MR	MR
7	Jul9809	HS	MS	S	MS	S	MS	MR	MR
8	Oct9806	HS	MR	MS	R	MR	R	MR	MR
	Jan9804	HS	MR	MS	R	MR	R	R	R
9	Oct9809	HS	MR	MR	MR	MS	MR	MR	R
10	Jul9803	S	MR	R	R	R	S	R	MR
11	Oct9804	HS	MS	R	MR	MS	MR	MR	R
12	Jul9813	HS	MR	S	MR	S	MR	MR	MR
	Jul9818	HS	MR	MS	MR	MS	MR	MR	MR
	Jul9814	HS	MR	MS	MR	S	MR	MR	R
13	Jul9805	HS	MR	S	MS	MR	MR	MR	R
14	Jul9804	HS	MS	MS	MR	MS	MR	R	R
	Feb9710	HS	MS	MS	MR	MS	R	MR	MR
15	Feb9923	HS	MS	MS	MR	MS	R	MS	R
	Nov9808	HS	MS	MS	R	MS	MR	MS	R

Based on a 0-9 scale where, 0,1 = resistant (R), 2,3 = moderately resistant (MR), 4,5 = moderately susceptible (MS), 6,7 = susceptible (S), and 8,9 = highly susceptible (HS).

Table 21. Pathotype variation of *Ascochyta rabiei* isolates from fields in Saskatchewan.

Location	Isolates	Pathotype	Location	Isolates	Pathotype
Demaine	Nov9806	1	Brock	Jul9818	12
	Nov9805	2	Cabri	Oct9806	8
	Nov9807	2	Dinsmore	Oct9805	3
	Nov9808	15	Elrose	Feb9914	2
			Elrose	Jul9808	5
Goodale[†]	Sep9804	6	Elrose	May9804	5
	Jul9805	13	Elrose	Jul9804	14
			Eston	Feb9803	3
Imperial	Oct9808	2	Eston	Jul9810	5
	Oct9809	9	Kyle	Dec9804	2
			Plato	Jul9809	7
Kyle	Jul9812	4	Rosetown	Nov9803	1
	Jul9811	6	Rosetown	Jan9907	2
	Jul9813	12	Sanctuary	Jul9814	12
			Simmie	Feb9923	15
Saskatoon	Jul9806	4	Sovereign	Jul9815	1
(U of S, Preston)	Jul9807	4	Sovereign	Jul9816	1
	Aug9803	4	Vanscoy	Oct9804	11
Saskatoon	Mar9870	2	May9804		
(AAFC farm)	Jan9702	4	x	Jan0001	3
	Jan9805	6	Jan9702		
	Jan9804	8			
	Feb9810	14			
Swift Current	Sep9806	2			
	Jul9803	10			

Fig. 3. Dendrogram of Canadian and foreign isolates of *Ascochyta rabiei* scored on eight chickpea differential lines, based on the unweighted pair-group method with arithmetic averages using average taxonomic distance. (AUS = Australia, IN = India, SY = Syria, TY, Turkey and USA = United States of America).



Population diversity of *Ascochyta rabiei* in Saskatchewan

Background: High levels of pathogenic variability in *A. rabiei* has been reported in many countries around the world countries; we identified 15 pathotype groups in Canada (see above). Several previous studies have used molecular techniques to investigate genetic diversity, relationships between molecular markers and pathogenicity, and to detect evidence of phylogenetic relationships for *A. rabiei*. Populations of *A. rabiei* can be highly variable even within a single field. However, in many studies, no correlations have been found between markers and pathotype groups or mating types in *A. rabiei*.

The objectives of this study were to: 1) assess genetic variability among *A. rabiei* isolates using RAPD's, 2) identify any correlations between RAPD makers with geographical origin and pathotype groups and 3) compare *A. rabiei* to *A. lentis*, *M. pinodes*, *A. candida* and *L. maculans*. Studies on population variations of *A. rabiei* in western Canada are important for understanding the genetic structure of the pathogen population, which is needed by breeding programs and will enhance the understanding of disease epidemiology and effective management of ascochyta blight on chickpea.

Materials and Methods: Isolates *of A. rabiei* from a wide range of locations were examined: 39 from Saskatchewan, 14 from crosses among four Canadian isolates (not discussed in the current report), and 20 from other countries (6 from Syria, 5 from Australia, 5 from USA. and 2 each from Turkey and India). In addition, two *M. pinodes*, seven *A. lentis* and three unidentified isolates of *Ascochyta* spp. from field pea were included for comparison with *A. rabiei*. As well, one isolate each of *Albugo candida* and *Leptosphaeria maculans* were also included for comparison. Each isolate was single spored and maintained at -80°C until required.

Each isolate was grown in a 100 mL flask containing 50 mL potato dextrose broth at room temperature. The flasks were placed on an orbital shaker at 100 rpm. Mycelia from 5-day-old cultures were harvested by filtration and stored in 2 mL vials at -80°C. When needed, mycelia were freeze-dried for three days after which two plastic beads (2 mm-dia.) were put in each sample vial and then vials were kept on a shaker for 15 min to pulverize the mycelia into fine powder. Total DNA was extracted using the CTAB method. Initially, 75 decamer primers (University of British Columbia, Vancouver, Canada) were screened, based on polymorphisms from 10 isolates. Eight of the primers showed good polymorphisms and were selected for screening the whole population of isolates (Table 22).

The DNA of each isolate was amplified in a total volume of 20 µL comprising 5.8 µL sterile double de-ionized H_2O, 2 µL 10x buffer, 2 µL 25 mM $MgCl_2$, 2 µL 2 mM dNTPs, 0.2 µL Taq DNA polymerase (5U μL^{-1}), 4 µL 1 mM primer and 4 µL 10 ng L^{-1} genomic DNA. Amplifications were performed using two thermal cyclers, each with an initial denaturation step for 5 min at 94°C, followed by 35 cycles of 30s at 94°C, 30s at 37°C and 30s at 72°C, with a final extension for 7 min at 72°C. Amplified products were separated by electrophoresis in 2 % agarose in 1x TAE buffer. For each primer and isolate combination, the amplification reactions were performed two times in each PCR machine to determine the reliability and reproducibility of the methods. Reactions were kept at 4°C until gel electrophoresis was performed. In each gel, a 1-Kb Plus DNA ladder was used as a molecular reference marker. Gels were stained in ethidium bromide for 20 minutes and then destained in tap water. Fragments of DNA were

visualized and photographed under transmitted ultra violet light in black-and white prints.

In cluster analysis, all eight primers were used on all 73 isolates of various species (excluding 14 isolates from crosses) which in combination, produced 112 bands. For each isolate and primer combination, a binary system was used to score the bands (0 = absence, 1 = presence, regardless of band intensity). This binomial data matrix was used to construct a similarity matrix using Nei's coefficient. The resulting similarity data was assessed by cluster analysis using the unweighted pair-group method with arithmetic averages. All calculations were made using the NTSYS-PC program (Exeter Software, USA).

Results: For ease of presentation, only representative banding patterns of selected isolates are presented. Isolates of *A. rabiei* from Canada produced at least seven different banding patterns (Figs. 4 & 5). However, about 30 isolates were of three characteristic banding patterns, represented by isolates 2, 6 and 25. All 5 isolates from Australia, 3 of 5 isolates from the USA and 3 of 6 Syrian isolates produced the same banding pattern, which was also the same as to that produced by one group of isolates from Canada represented by isolate 2. Isolates from India and Turkey displayed banding patterns that were clearly distinct from patterns produced by isolates from any other country (Figs. 4 & 5).

In cluster analysis, all of the non-*A. rabiei* isolates clustered at one end (Fig. 6). The isolates from Australia, three from the USA, both isolates from Turkey and two groups of isolates from Syria formed sub-clusters within the *A. rabiei* group. The difference among the most two dissimilar *A. rabiei* isolates was about 42%. Isolates of *A. lentis* and *M. pinodes* were clustered in individual groups by species, but *A. lentis* was more closely related to *A. rabiei* (Fig. 6). In general, isolates of *M. pinodes*, *A. candida* and *L. maculans* clustered at a higher distance from *A. rabiei* than *A. lentis*.

Discussion: The assessment of genetic variation in isolates of *A. rabiei* from Canada and other countries with eight decamer primers show substantial genetic variation in this pathogen. This variation was detected among isolates from different countries and also from within local populations of isolates from each country, with the exception of isolates from Australia, which all exhibited one type of banding pattern. Genetic diversity was high in isolates from Canada compared to isolates from other countries. This can be attributed to the larger population size of Canadian isolates relative to samples from other countries. For each primer, several amplification fragments were observed to be common among the majority of *A. rabiei* isolates. Some of these specific bands could be used as characteristic genetic markers for identifying *A. rabiei* isolates.

The majority (65%) of Canadian isolates exhibited three characteristic banding patterns. The rest of the isolates where represented by five other patterns. In comparing results of the present study to those of the pathotypes study (described above), there was no correlation between pathotype groups and RAPD markers. This lack of correlation between RAPD groups and pathogenicity has been reported previously in *A. rabiei*.

Chickpea is a recent introduction into both Canada and the USA and *A. rabiei* is believed to have been introduced into North America on infected seed. Similarities in the banding pattern of some Canadian isolates and those from the USA, Australia, Syria and India support this hypothesis. A specific amplification pattern observed in some isolates from Canada and the USA was similar to a that of certain isolates from Syria, India and Australia. It is likely then that the

population in Canada and the USA originated from Syria or India. The pathogen may also have been introduced into Australia via seed imports from India or Syria.

Table 22. Sequences of 10-mer RAPD primers used.

Primer	Nucleotide sequence 5' to 3'
UBC-702	GGG AGA AGG G
UBC-708	GGG TTG TGG G
UBC-720	GGG AGG GAG A
UBC-725	GGG TTG GGT G
UBC-726	GGT GTG GGT G
UBC-727	GGG TGT GGT G
UBC-731	CCC ACA CCA C
UBC-739	GGA GGG AGA G
UBC-740	GGA GGG AGG A
OP-C18	TGA GTG GGT G

Fig. 4. RAPD banding patterns of representative samples of *Ascochyta rabiei* (lanes 2-17), *Ascochyta lentis* (*A.l.*, lanes 18-19), *Mycosphaerella pinodes* (*M.p.*, lane 20) and *Ascochyta* spp. (*A.*, lane 21) isolates generated by primer UBC 702. M = molecular marker.

Fig. 5. RAPD banding patterns of representative samples of *Ascochyta rabiei* (lanes 2-17), *Ascochyta lentis* (*A.l.*, lanes 18-19), *Mycosphaerella pinodes* (*M.p.*, lane 20) and *Ascochyta* spp. (*A.*, lane 21) isolates generated by primer UBC 727. M = molecular marker.

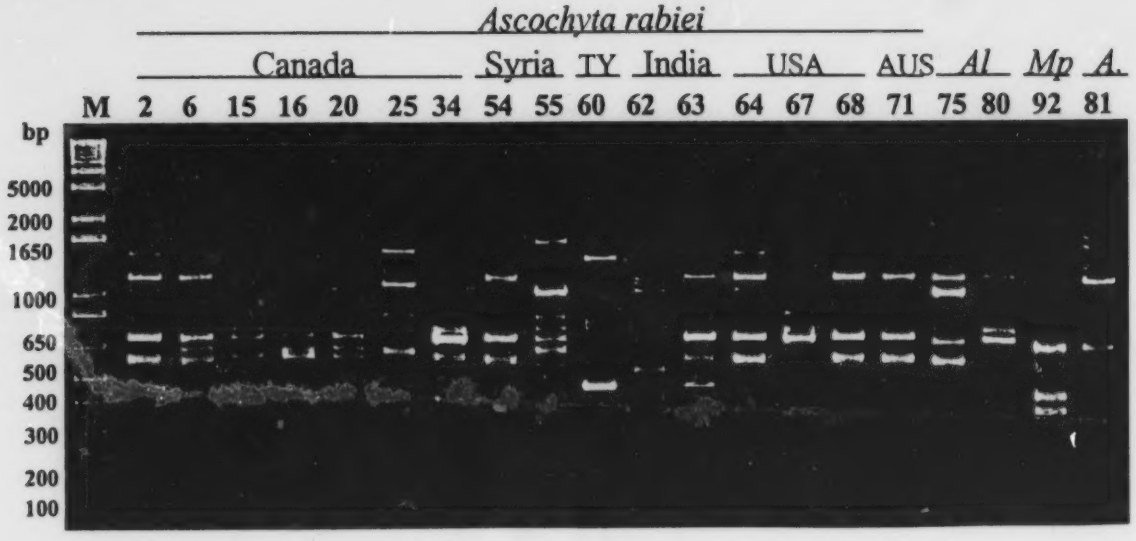
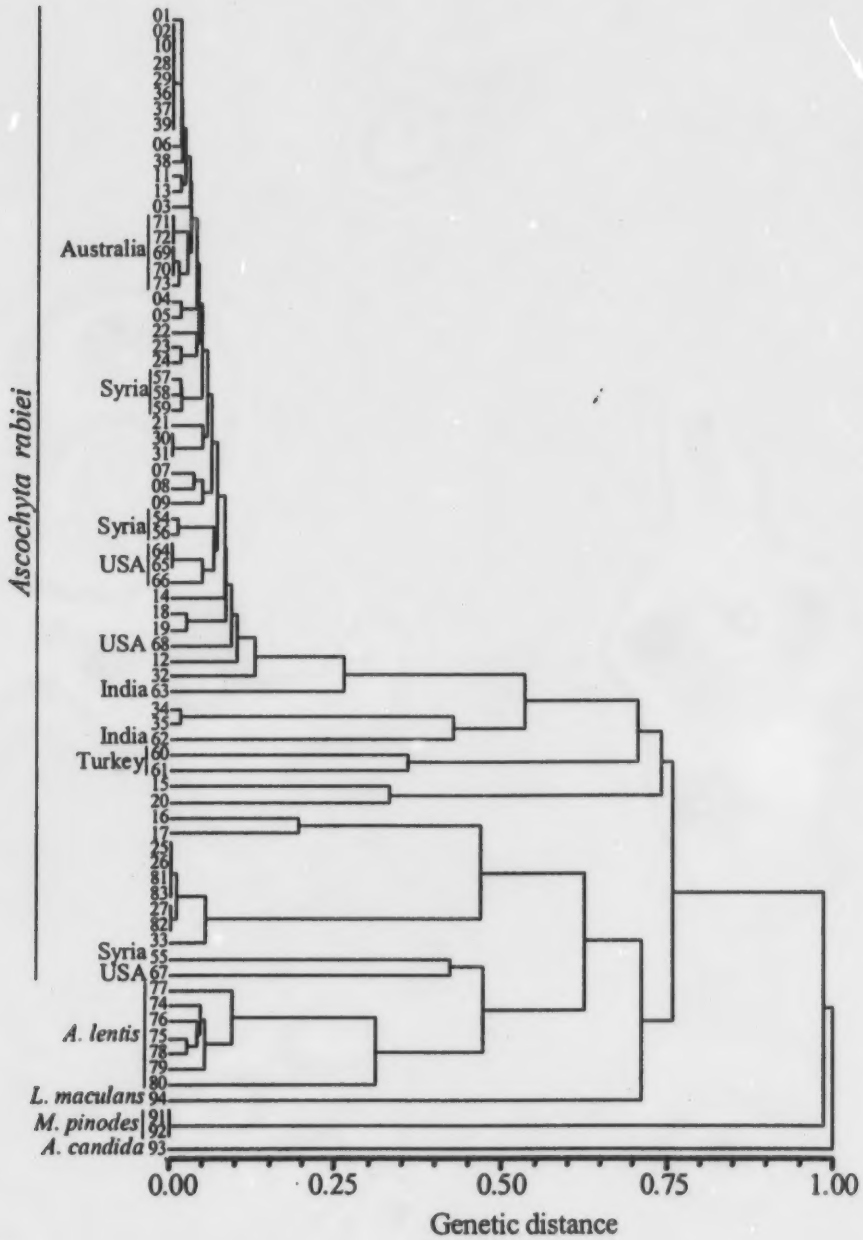


Fig. 6. Dendrogram showing relationship among isolates of *Ascochyta rabiei* from Canada and in relation to *Ascochyta lentis*, *Mycosphaerella pinodes*, *Albugo candida* and *Leptosphaeria maculans*.



OTHER STUDIES

Background: The impact of anthracnose on lentil production in Saskatchewan has increased rapidly in the last few years, and no resistant cultivars are available. In response, several studies were undertaken as part of this research study, to examine the biology and management of anthracnose on lentil. Drs. Buchwaldt and Chongo are experts on anthracnose, and we have built on their experience and knowledge to rapidly expand our involvement in this area in response to the increased importance of anthracnose in the region. A graduate student, Krista Anderson, is participating in many of these projects. In addition, the importance of seedling blights and root rots of pulses is increasing as they become a common part of the cropping rotations across the prairies. Several studies were undertaken to examine the biology and control of this poorly understood group.

Disease surveys

Disease surveys on lentil and chickpea in Saskatchewan were conducted in 1998 and 1999. Anthracnose on lentil was found in half of the field in 1998 and in 1/3 of the fields in 1999; it was severe in some areas in each year. Inoculum of anthracnose can be moved over long distances by wind, so the disease can spread quickly. This information was provided immediately to extension specialists and producers via the website, and was subsequently published in the Pesticide Management Research Report to alert other specialists to the spread of this pathogen in the region.

Disease surveys on chickpea showed that the disease had moved into all of the major chickpea producing areas of the province. Again, extension specialists and growers were alerted, allowing many producers to take timely action to control this disease in their crops. In several instances, the sudden increase in demand for fungicides caught suppliers across the region by surprise. However, most growers are now familiar with the symptoms of blight and suppliers are maintaining larger supplies to meet the potential demand.

Infection process of anthracnose

Background: *Colletotrichum truncatum* causes anthracnose, which is the most damaging disease of lentil in western Canada. Current disease management practices are based primarily on application of foliar fungicides, because no commercial cultivars are resistant. The process of infection by *C. truncatum* has been described on pea, soybean and hemp sesbania, but the process is not the same in these three hosts. The objective of this study was to investigate the response of partially-resistant and susceptible lentil genotypes to infection by *C. truncatum*, from the initial phase of the infection process until lesion formation. This information will contribute to our understanding of the basis for genetic resistance in lentil and taxonomic relationships in this problematic genus.

Materials and Methods: The study was run as a 2 x 2 factorial, with two isolates of *C. truncatum* as one factor and two lentil lines as the second factor in a randomized complete block design with three replicates. Isolates JPPTNL 882 and 95S29 were selected to represent different levels

of aggressiveness on lentil. Of 27 isolates of *C. truncatum* collected from lentil in Manitoba between 1993 and 1996, 95S29 was the most virulent isolate and JPPTNL 882 was in the intermediate range. The partially resistant lentil line PI 320937 (Bernier et al. 1992) and the susceptible cv. Eston were selected to represent the range of resistance currently available. The experiment was repeated 10 times.

Seed of each cultivar was sown in 0.5 L plastic pots containing a soilless potting medium, with 5 plants per pot. The plants were maintained in a growth cabinet at 20:16°C (day:night) and 16 h photoperiod. A balanced fertilizer was supplied as required. Single-spore cultures of both isolates were grown on sodium-chloride yeast sucrose agar at room temperature under continuous fluorescent light. Plants were inoculated at 4 wk after seeding (8-10 node stage) with 5-6 mL of a conidial suspension (4×10^4 conidia mL^{-1}) per pot using an atomizer. Inoculated and noninoculated plants were exposed to a 24 h continuous wetness period in a mist chamber.

Leaflets from inoculated and noninoculated plants were sampled at 1, 3, 6, 12, 24, 48, 72, 96, 120 and 144 h post-inoculation (hpi). At each sampling period, 10 leaflets per replicate were sampled at random from each genotype, making a total of 30 leaflets, and cleared in a solution of glacial acetic acid and absolute ethanol (1:2). The fixative was changed once after 24 h. Samples were mounted on glass slides in lactophenol containing 0.05% aniline blue. Conidial germination was assessed microscopically on each sample. The number of internal hyphal colonies (HC) was counted, the colony diameter (CD) was measured on each leaflet, and the number of visible lesions (LN) per leaflet were counted. At 144 hpi, the size of each leaflet and each lesion was determined using a computer imaging system (Chongo and Bernier 1999). The number of hyphal colonies and lesions on leaflets were then converted to numbers per cm^2. Colonization efficiency (CE) was calculated as the ratio of the number of lesions to number of hyphal colonies (LN:HC) per cm^2 of leaf area at 144 hpi. Stem samples were also collected at each sampling time. Cross and longitudinal stem sections were cut (freehand, or with a microtome on samples embedded in paraffin wax), then cleared and stained as described above.

The growth pattern of hyphae in whole leaves and in stem sections was observed using bright field microscopy. In addition, phenolic compounds were visualized by staining with toluidine blue in a citrate buffer at pH 4.0, in fresh and cleared samples. The presence of lignin was assessed using phloroglucinol dissolved in 20% hydrochloric acid, which stains lignin red. Tissues of cv. Eston inoculated with isolate 95S29 were assessed to determine the viability of infected cells. Longitudinal stem sections or strips of the epidermis were placed in a plasmolysing solution of either 0.85 M $NaNO_3$ or KNO_3 containing 0.01% (w/v) neutral red and the samples were viewed using bright field microscopy.

Data for conidial length and width were analysed using a t-test. Analysis of variance was used for number HC cm^{-2}, CD, LN cm^{-2} , LS and CE on leaflets using the GLM procedure of SAS.

Results and Discussion: The taxonomy of *Colletotrichum* spp. is based on host range and conidial morphological characteristics. In previous studies (our lab and others), isolates of *C. truncatum* from lentil and pea infected and sporulated on both hosts, but did not infect soybean. When the conidia germinated in distilled water, a single septum developed in the middle of each conidium prior to the emergence of germ tubes. At 2 - 4 h, one to three germ tubes emerged from each conidium, either terminally or sub-terminally. Germ tubes usually

developed a sessile appressorium at the tip between 12 - 24 h, but some appressoria were non-sessile. Appressoria generally became more abundant and more melanized by 48 h (Fig. 8A). We observed that infection pegs often penetrated into other appressoria and conidia. This may represent a mechanism for genetic exchange through hyphal fusion, but more detailed studies are required to confirm this hypothesis.

When conidia of *C. truncatum* were inoculated onto lentil, infection of both isolates was similar on both genotypes between 1 and 48 hpi. Germination occurred within 3-6 hpi and appressoria began to form at 6-12 hpi. Each conidium produced one or two germ tubes, and one or two appressoria at the tip of each germ tube. By 24 hpi, infection pegs were observed in epidermal cells and intracellular primary hyphae had began to develop (Fig. 7B). Direct penetration of leaf and stem cuticles by infection pegs was the only method of infection observed on lentil, as has been reported for pea. After penetration, intracellular hyphal grew extensively in the colonized epidermal cells and then spread through the surrounding tissue, but inoculated plants remained symptomless for at least the first 48 hpi.

Differences in infection among treatments became apparent after 48 hpi. Lesions became visible between 72 to 144 hpi in the susceptible cv. Eston and up to 14 days after inoculation in the resistant line PI 320937. Initially, the lesions appeared light greenish and water soaked, then became necrotic. Infected cells of Eston (and non-infected cells) plasmolysed and accumulated neutral red. This shows that *C. truncatum* initially has a biotrophic relationship with the lentil host; a symptomless infection of host cells lasting at least 2 days is followed by extensive cell damage and lesion formation.

Hyphae of the moderately aggressive isolate JPPTNL 882 proliferated in leaf and stem tissue of Eston (Fig. 7C). The lateral expansion of hyphal growth was restricted on PI320937 (Table 23). Growth was often limited to hyphal aggregations (Fig. 7D), which became more numerous between 72 and 144 hpi. This was associated with production of fewer, smaller lesions on PI 320937 than on Eston (Table 23). For the aggressive isolate 95S29, no aggregations were observed in PI 320937, but hyphae were narrower in diameter than in Eston. We are confident that the aggregations in PI 320937 were fungal in origin. Sequential sampling of infected plants showed a progression from very early samples with spreading hyphae to development of aggregations. The fact that these aggregations occurred in the resistant line, but not the susceptible one, may indicate that they are associated with a host defence response.

Both lentil lines produced lignin in response to infection, based on red staining of host tissues treated with phloroglucinol. Similarly, both lentil lines produced a positive reaction to toluidine blue, indicating the accumulation of phenolic compounds; hyphae and cytoplasmic contents in leaflets turned bluish-purple in Eston and brownish in PI 320937, and stem sections of PI 320937 were a deep blue. Many phenolic compounds are toxic to microorganisms and production of phenolic compounds by host tissues has been associated with a resistance response.

Colletotrichum species are well known for their ability to colonize host tissue without provoking host defences. In the present study, the majority of infections were symptomless for the duration of the study and did not proceed to the necrotic stage that results in lesion formation. There were no differences between the isolates for colonization efficiency, but both isolates colonized tissues of Eston more efficiently than PI 320937 (Table 23). The highly aggressive isolate 95S29 consistently produced more numerous, but smaller hyphal colonies on leaflets than isolate JPPTNL 882. Both isolates produced more and larger colonies on Eston than PI 320937.

The same pattern was observed for size and frequency of lesions (Table 23). Occasionally, hyphal colonies merged together, resulting in larger lesions.

We noted consistent differences in morphology, host range, and infection process between isolates of *C. truncatum* from lentil and pea compared to previous reports of isolates from soybean. This indicates that further work is required to clarify their taxonomic relationship. In addition, the process of infection by *C. truncatum* varied, depending on the resistance reaction of the host line and the aggressiveness of the pathogenic isolate, from hyphal aggregations and restricted hyphal spread to expanding lesions.

This information has been prepared for publication, and a scientific paper by Chongo et al. describing the work is currently in review.

Table 23. Number of hyphal colonies (HC), colony diameter (CD), colonization efficiency (CE), lesion number (LN) and size (LS) on leaflets of lentil lines Eston and PI 320937, 144 h after inoculation with one of two isolates of *Colletotrichum truncatum* (n = 30 leaflets).

		Characteristic				
Line	Isolate	HC (cm^{-2})	CD (μm)	LN (cm^{-2})	LS (mm^2)	CE[1]
Eston	JPPTNL 882	20.6	418	6.4	2.5	0.31
	95S29	25.4	348	8.8	2.1	0.35
PI320937	JPPTNL 882	17.5	292	2.6	0.6	0.15
	95S29	24.7	223	5.6	2	0.23
$LSD_{0.05}$		4.2	68	2.3	1.1	0.09

[1]CE = Ratio of LN : HC cm^{-2} on leaflets.

Fig. 7. Light micrographs of *Colletotrichum truncatum* (isolate JPPTNL 882), showing: **A)** germ tubes with appressoria on a glass slide 48 h after mounting, (a = appressorium); **B)** a stem cross section of lentil cv. Eston with hyphae in an epidermal cell, stained with toluidine blue at 24 h post inoculation (hpi); leaflets stained with aniline blue, with **C)** unrestricted hyphal growth on Eston at 48 hpi; and **D)** hyphal aggregations in resistant line PI 320937 at 72 hpi. Bar = 50μm.

Pathotypes of anthracnose

Background: During the course of several recent studies to identify sources of resistance to anthracnose in lentil, isolates have been identified that are pathogenic on the few resistant lines that are available. Work was undertaken to determine if these unusual isolates represent different races of the pathogen, and their frequency in the population in Manitoba and Saskatchewan.

Results and Discussion: Two races of anthracnose were identified from western Canada. A few lentil lines have been identified that are resistant to Race 1, but none to Race 2. Race 2 is the most common race in western Canada, so it represents a continuing threat to lentil production in this region. The results of this work have been written up for publication, and the draft text is currently in a review prior to submission for publication (Buchwaldt, L., Anderson, K.L., Morrall, R.A.A., Bernier, C.C. and Gossen, B.D. 200x. Screening of lentil for resistance to *Colletotrichum truncatum* and identification of two pathotypes.). A draft version of the paper is attached as Appendix 4.

Host range of anthracnose

Background: The pathogen that causes anthracnose of lentil is relatively new to the region and is poorly understood. One critical aspect of disease control is knowing where the reservoirs of infection may occur. To partially fill this information gap, we continued studies that had been initiated at the U of M to assess the host range of *C. truncatum* in greenhouse and field trials. This study, together with several others, including examining the genetic variability of this pathogen using molecular techniques, were undertaken as part of the M.Sc. research program of a graduate student (K. Anderson). Ms. Anderson expects to complete her studies by spring of 2002, but has already published several abstracts of her work in scientific journals.

Seedling blight and root rot of pulses

Background: Seedling blight and root rot of pulse crops has been identified as an increasing problem in the prairie region, as the become an increasingly important part of the production system. A number of studies on seedling and root pathogens of pulses were conducted in cooperation with Drs. S.F. Hwang in Alberta Research Council.

Results and Discussion: A number of these studies have been published and reprints are included as Appendices 5 - 7. They include:

Hwang, S.F., Gossen, B.D., Turnbull, G.D., Chang, K.F., Howard, R.J. and Thomas, A.G. 2000. Seeding date, temperature, and seed treatment affect pythium seedling blight of field pea. Can J. Plant Pathol. 22: 392-399.

Hwang, S.F., Gossen, B.D., Turnbull, G.D., Chang, K.F., Howard, R.J. and Thomas, A.G. 2000. Effect of temperature, seeding date, fungicide seed treatment and inoculation with *Fusarium avenaceum* on seedling survival, root rot severity and yield of lentil. Can. J. Plant Sci.

80: 899–907.

Hwang, S.F., Gossen, B.D., Chang, K.F., Turnbull, G.D. and Howard, R.J. 2001. Effect of seed damage and metalaxyl seed treatments on pythium seedling blight and yield of field pea in Alberta. Can. J. Plant Sci. 81: 509-519.

Chongo, G., Gossen, B.D., Kutcher, H.R., Gilbert, J., Turkington, T.K., Fernandez, M.R. and McLaren, D. 2001. Reaction of seedling roots of 14 crop species to *Fusarium graminearum* from wheat heads. Can. J. Plant Pathol. 23: 132-137.

These studies show that seedling blight and root rot can result in substantial yield and quality loss, and can result in patchy stands that are difficult to harvest and susceptible to weed invasion. Cracked or damaged seed is especially susceptible to seed rot damage. The studies also identified disease management strategies, that include crop rotation, early seeding, vigorous seed and seed-treatment fungicides for situations where pathogen populations in individual fields are high. Techniques developed in these studies were used to examine the reaction of a range of common crop plants to the pathogen that causes fusarium head slight in wheat.

Additional scientific papers on the impact of seedling blight on chickpea, and on the reaction of lentil cultivars to fusarium root rot are in preparation, but much of this information is already available to growers in factsheets and newsletters, or on the website.

Miscellaneous

The Lentil Co-op trials (conducted by the Prairie Regional Recommending Committee on Grain) were assessed for disease each year. Lines in these trials were also screened under controlled conditions for resistance to specific isolates of anthracnose and ascochyta blight. Recommendations for support of several lentil lines for registration were made to the PRRCG.

Technology transfer

One of the strongest points of this program has been the ongoing commitment of every member of the team to participate in extension and technology transfer. The extent of that commitment is clearly evident from the list of tech transfer publications and invited presentations at the end of this section. However, the two items below merit special attention.

Establishment of a web site on "Pulse Crop Diseases": A 40-page website was launched in May 1999 at http://paridss.usask.ca/specialcrop/pulse_diseases. The site has three components: 1) symptoms, disease cycles and disease management options for mycosphaerella blight on field pea, anthracnose and ascochyta blight on lentil, and ascochyta blight on chickpea, 2) fungicide decision support systems in lentil and pea, and 3) real-time reports on disease outbreaks of pulse diseases in Saskatchewan during the growing season, provided by staff with SK Agric & Food and AAFC. Three new sections were added in May 2000: powdery mildew of pea, root rot of pea, and botrytis blight on lentil, and the site was updated again in 2001. Programming of the website was carried out by Northern Lights Internet Solutions of Saskatoon. The site is hosted on the AFIF Spoke website server. Since its inception, this website has had from 100-700 hits per day, with about 80% of the hits from within Canada. This has been an outstanding tool for making research information available to large numbers of extension specialists and producers.

Demonstrations at AFIF Spoke sites: Fungicide demonstration trials in lentil, pea and chickpea was conducted at seven AFIF Spoke sites in 1999 and 2000. These trials were planted, maintained and harvested by the site managers; trained personnel from our program carried out three disease ratings at each location. Some sites were not harvested due to weeds and other problems, but most of the sites provided information that fed directly into the fungicide and decision support studies, while providing growers with an opportunity to see the research study in action in their local area.

Technology transfer publications

1) Kutcher, R. and Gossen, B.D. 1998. Pea disease control trial. Pg. 8-9 in Field Report 1997, Saskatchewan Conservation Learning Centre, Prince Albert, SK.
2) Xue, A, Warkentin, T., Rashid, K., Gossen, B., Burnett, P., Vandenberg, A. 1998. Study boosts disease resistance breeding program for peas. Western Grains Res. Foundation, Industry Report, Sept. 16, 1998
3) Buchwaldt, L., 1999. Handling of anthracnose-infected lentil stubble: Avoid tillage. Saskatchewan Pulse Grower 15 (3): 15.
4) Buchwaldt, L. A new website on Pulse Crop Diseases, Scientific authority, SAF Farm & Food Report, 99-25-123.
5) Buchwaldt, L. 1999. Emphasis on lentils. Scientific authority, Top Crop Manager, April 1999.
6) Buchwaldt, L. and Denys, S. 1999. Tips for anthracnose and ascochyta control in lentils. The Western Producer, Farming. Vol. 2 (6): 27-28.
7) Buchwaldt, L. and Gossen, B.D. 1999. Pulse disease website. (http://paridss.USask.Ca/specialcrop/pulse_diseases/index.html).

8) Buchwaldt, L. and Gossen, B.D. 1999. When to spray fungicides in lentil and pea crops. SK Pulse Grower 15(2):1,6-7.
9) Buchwaldt, L. and Gossen, B.D. 1999. Annual Report 1998, AFIF Project #96000300: Integrated disease management for chickpea, lentil and field pea. 11 pp.
10) Buchwaldt, L. Chongo, G. and Gossen, B. 1999. Management of lentil anthracnose. AAFC, Saskatoon Research Centre Factsheet, 2 pp.
11) Buchwaldt, L. 2000. Should you spray for anthracnose? Scientific authority, Country Guide, April 2000.
12) Buchwaldt, L. and Gossen, B.D. 2000. When to spray fungicides in lentil. The SK Pulse Grower 16(2):16-17.
13) Buchwaldt, L., Kutcher, H.R. and Gossen, B. 2000. Effect of foliar fungicide application on control of mycosphaerella blight in pea 1999. Field Report 1999, Saskatchewan Conservation Learning Centre, Prince Albert, SK.
14) Chongo, G., Gossen, B.D., Armstrong, C. and Buchwaldt, L. 2000. "Resistant" chickpeas are susceptible to ascochyta blight at flowering. Res. Letter 00-04, Saskatoon Res. Cen, AAFC, Saskatoon, SK. 2 pp. (Also published in The SK Pulse Grower 16(2) :22).
15) Gossen, B.D. 2000. Pulse disease research. Pg 7 in Tour program, 6th Intern. Symposium on the Biosafety of Genetically Modified Organisms, Saskatoon, SK July 10, 2000.
16) Buchwaldt, L., and Gossen, B.D. 2001. To spray or not to spray: assessing disease risk in lentil. The SK Pulse Grower 17 (2): 1-4.
17) Buchwaldt, L., Gossen, B.D., and Pearse, P. 2001. Ascochyta blight of lentil. Research Letter 03-01, May 22, 2001. Saskatoon Res. Cen., AAFC, Saskatoon. SK.
18) Gossen, B.D. 2001. Use every defence against ascochyta in chickpeas. Scientific authority for article by Helen McMenamin in Top Crop Manager, February 2001, pg. 108-109.
19) Gossen, B.D., Hwang, S.F., and Pearse, P. 2001. Seedling blights of pulses. Research Letter 01-01, May 4, 2001. Saskatoon Res. Cen., AAFC, Saskatoon. SK.
20) Gossen, B.D., Buchwaldt, L., Chongo, G., Armstrong, C., and Pearse, P. 2001. Ascochyta blight of chickpea. Research Letter 02-01, May 11, 2001. Saskatoon Res. Cen., AAFC, Saskatoon. SK.
21) Pearse, P., McVicar, R., Goodwillie, D., Gossen, B., Buchwaldt, L., Chongo, G. and Armstrong, C. 2001. Farm Facts: Ascochyta blight of chickpea. Saskatchewan Agriculture and Food, Regina. SK. 4 pp.

Invited Presentations

- Field Day, Saskatchewan Conservation Learning Centre, Prince Albert, SK July 24, 1997.
- SK Soil Conservation Assoc. meeting, Rosetown, SK, January 30, 1998.
- SK Pulse Growers annual field day at Indian Head, July 15, 1998
- Gustafson dealers and service reps, Saskatoon, December 4, 1998.
- Seed treatment licencing workshop, organized by SIAST, Saskatoon, December 10, 1998.
- SAF/SPG grower meeting, Davidson, Kindersley, Elrose and Conquest, 1999.
- Grower meeting, seed treatment and disease control, Demaine SK on March 1, 1999.
- SAF/SPG grower meeting, Management of pulse diseases, Watrous SK, March 5, 1999.
- Grower meeting, disease control in pulse crops, Eston SK, March 23, 1999.

- Saskatchewan Pulse Grower's (SPG) annual meeting, Saskatoon, January 11, 2000.
- CSGA Select Grower Meeting, Saskatoon, January 12, 2000.
- SAF grower meetings, Elrose & Kindersley, January 20, 2000.
- SAF grower meetings, Outlook & Davidson, January 21, 2000.
- SAF/SPG grower meeting, Shaunavon, January 31, 2000.
- SAF/SPG grower meeting, Assiniboia, February 1, 2000.
- SAF/SPG grower meeting, Rosetown, February 7, 2000.
- SAF Extension Division, Agronomy Workshop at U of S, Saskatoon, February 22-23, 2000
- Panel discussion at Soils and Crop 2000, February 24, 2000.
- SAF grower meeting, Harris, March 8, 2000.
- Zeneca Agro Sales Manager's meeting, Regina, April 5, 2000.
- Saskatchewan Crop Insurance Crop Adjuster's meeting, Saskatoon, May 10, 2000.
- Co-op Hail Insurance Crop Adjuster's meeting, Regina, June 3, 2000.
- SPG/SAF pulse grower's field day, Swift Current, June 6, 2000.
- SPG/SAF pulse grower's field day, Redvers, August 1, 2000.
- AFIF conference, Regina, November 8, 2000.

PERFORMANCE INDICATORS

Impact

The research program produced results that are relevant to producers. In order to get these results out into the field, extension was an important component of our activities (see above). Articles were published in grower magazines, factsheets and newsletters were published, and numerous presentations were made at producer meetings and field days throughout the province, and the web site received 100-700 hits per day ever since its inception. These diverse approaches help us maintain contact with a wide range of pulse producers. For example, the presentation at Pulse Days 2000 was attended by 1200 people. More than 10,000 copies of one factsheet were purchased by government and industry groups to distribute to their clients in the last three years, and several new factsheets on disease management in pulses were prepared and distributed across western Canada early in the 2001 growing season.

This research has stimulated a lot of interest among provincial extension staff, who are recommending the decision support system to lentil growers and using our results to make fungicide application recommendations for chickpea producers. Also, based on the results of this work, Quadris was granted emergency registration on chickpea in 2000 and 2001, and the company is proceeding with full registration for all of the pulses. As a result of these efforts, growers have been able to manage to increased risk of disease in lentil caused by the rapid expansion of anthracnose disease with relative ease. However, the most important indicator of impact of this research on pulse producers in this province is that the acreage of chickpea has continued to increase, despite several years where conditions for the development of ascochyta

blight were excellent. We provided timely recommendations on rates, timing and economics of fungicide application for chickpea, identified that wind-borne ascospores can infect otherwise clean fields, provided recommendations for cropping intervals to reduce pathogen populations, and even developed recommendations for control of seedling blights.

Interaction with outside scientists is maintained through presentations at provincial, national and international meetings, and publications in scientific journals. So far, scientists in this program have published 11 scientific paper (+ 4 in review), 21 reports, 24 abstracts, 21 tech transfer articles, a M.Sc. thesis, an invited conference presentation and a book chapter.

Scientists in the project participated in the revision of the Pulse Production Manual by providing materials and reviewing the sections on agronomy and disease management. The revised version was published in 2000 by the Saskatchewan Pulses Growers, and is widely used by top producers.

Benefits

The information developed in this research program is being used by growers to improve disease diagnosis in lentil, chickpea and pea, to improve the timeliness and necessity for fungicide application, and to reduce yield and quality losses caused by these diseases. Reducing losses from disease improves profitability and income stability for individual growers and the industry as a whole. We have also greatly expanded our understanding of the biology of both ascochyta blight of chickpea and anthracnose of lentil. As a result of this increased understanding, we have alerted plant breeders to the problems with breakdown of resistance that is currently looming over the chickpea industry, and investigated several approaches for dealing with this issue.

Human resources

Two master students have worked with our program; C. Armstrong, who recently completed her degree, worked on the biology of ascochyta blight of chickpea, and K. Anderson is completing studies on the biology of anthracnose in lentil. Dr. Chongo, who worked for three years as a Post-Doctoral Fellow, is recognized as an expert on *Ascochyta rabiei*, and has recently published several articles in refereed journals on related topics. He is currently employed in the Plant Sciences Department, U of S. Dr. Buchwaldt has become a well-known speaker at pulse growers meetings throughout the region, and has obtained funding for an independent project on marker-assisted selection for disease resistance in lentil. She is in the process of publishing several important papers on the develop of the DDS, and on race identification in *C. truncatum*, which will enhance her international reputation in this area. She is currently employed at AAFC, Saskatoon Research Centre. In addition, of the three technicians that were funded by this program, two have gone on to obtain secure employment in other research facilities, and one has gone on to graduate school.

New technology

1) The fungicide decision support system in lentil is a new tool for producers.
2) A set of chickpea differentials was developed and used to characterize races of *A. rabiei* in western Canada. This system will allow breeders to screen chickpea germplasm against the appropriate pathotypes of ascochyta for the region.
3) A new race of Colletotrichum truncatum was identified, and its distribution assessed.
4) An innovative feature on the Pulse Crop Diseases website is on-line reporting of diseases observations in the growing season. This information is collected by specially-trained field scouts, and is available to growers for use in planning and timing fungicide applications. Unfortunately, this feature was not continued when the site was taken over by Saskatchewan Agriculture and Food.
5) Syngenta (formerly Zeneca Agro) is using our evaluations of Quadris to obtain registration of this new and highly effective product in chickpea, lentil and pea. This fungicide will be a useful addition to the disease management arsenal of growers, because it provides effective disease control and also has a systemic effect, i.e. it controls disease on the foliage after infection has occurred. Positive results from Bravo Ultrex also provide growers with secure access to chlorothalonil, in the event that Bravo 500 is deregistered.

6) **Publications**:

a) Scientific journals

1) Xue, A.G., Warkentin, T.D., Gossen, B.D., Burnett, P.A., Vandenberg, A., and Rashid, K.Y. 1998. Pathogenic variation of western Canadian isolates of *Mycosphaerella pinodes* on selected *Pisum sativum* genotypes. Can. J. Plant Pathol. 20: 189-193.
2) Chongo, G., Bernier, C. C., and Buchwaldt, L. 1999. Control of anthracnose in lentil using partial resistance and fungicide applications. Can. J. Plant Pathol. 21: 16-22.
3) Chongo, G., and Bernier, C.C. 1999. Field and growth chamber evaluation of components of partial resistance to *Colletotrichum truncatum* in lentil. Can. J. Plant Pathol. 21:58-63.
4) Bailey, K.L., Johnston, A.M., Kutcher, H.R., Gossen, B.D. and Morrall, R.A.A. 2000. Managing crop losses from foliar diseases with fungicides, rotation, and tillage in the Saskatchewan Parkland. Can. J. Plant Sci. 80: 169-175.
5) Chongo, G., and Bernier, C.C. 2000. Effects of inoculum concentration, wetness period, growth stage and temperature on anthracnose of lentil. Plant Dis. 84: 544-548.
6) Hwang, S.F., Gossen, B.D., Turnbull, G.D., Chang, K.F., Howard, R.J. and Thomas, A.G. 2000. Seeding date, temperature, and seed treatment affect pythium seedling blight of field pea. Can J. Plant Pathol. 22: 392-399.
7) Hwang, S.F., Gossen, B.D., Turnbull, G.D., Chang, K.F., Howard, R.J. and Thomas, A.G. 2000. Effect of temperature, seeding date, fungicide seed treatment and inoculation with *Fusarium avenaceum* on seedling survival, root rot severity and yield of lentil. Can. J. Plant Sci. 80: 899–907.
8) Armstrong, C.L., Chongo, G., Gossen, B.D., and Duczek, L.J. 2001. Mating type distribution

and incidence of the teleomorph of *Ascochyta rabiei* (*Didymella rabiei*) in Canada. Can. J. Plant Pathol. 23: 110-113.

9) Chongo, G. and Gossen, B.D. 2001. Plant age affects resistance to *Ascochyta rabiei* in chickpea. Can. J. Plant Pathol. 23: xxx-xxx. (In press).
10) Chongo, G., Gossen, B.D., Kutcher, H.R., Gilbert, J., Turkington, T.K., Fernandez, M.R. and McLaren, D. 2001. Reaction of seedling roots of 14 crop species to *Fusarium graminearum* from wheat heads. Can. J. Plant Pathol. 23: 132-137.
11) Hwang, S.F., Gossen, B.D., Chang, K.F., Turnbull, G.D. and Howard, R.J. 2001. Effect of seed damage and metalaxyl seed treatments on pythium seedling blight and yield of field pea in Alberta. Can. J. Plant Sci. 81: 509-519.
12) Buchwaldt, L., Anderson, K.L., Morrall, R.A.A., Bernier, C.C. and Gossen, B.D. 200x. Screening of lentil for resistance to *Colletotrichum truncatum* and identification of two pathotypes. Phytopathology xx: xxx-xxx (internal review).
13) Buchwaldt, L., Chongo, C., Gossen, B.D., Morrall, R.A.A., Wahab, J., Bernier, C.C., and Rimmer, S.R. 200x. Development of a fungicide decision support system for control of anthracnose and ascochyta blight of lentil. Can. J. Plant Pathol. xx:xxx-xxx (in review).
14) Chongo, C., Gossen, B.D., and Bernier, C.C. 200x. Infection process of *Colletotrichum truncatum* in partially resistant and susceptible lentil genotypes. Can. J. Plant Pathol. xx:xxx-xxx (submitted).
15) Chongo, G., Gossen, B.D., Buchwaldt, L., Adhikari, T. and Rimmer, S.R. 200x. Genetic diversity of *Ascochyta rabiei* of chickpea in western Canada, assessed using RAPDs and pathogenicity tests. Can. J. Plant Pathol. xx: xxx-xxx (submitted).

b) **Books, Chapters, Conference Proceedings**

1) Armstrong, C.L. 2001. Ascospore production and infection of chickpea by *Ascochyta rabiei*. M.Sc. Thesis, University of Saskatchewan, Saskatoon, SK.
2) Chongo, G. and Gossen, B.D. 2002. Diseases of chickpea. Pg. xx-xxx in Diseases of Field Crops in Canada. Bailey, K.L., Gossen, B.D., Morrall, R.A.A. and Gugel, R.(eds.). Canadian Phytopathological Society. (Submitted)
3) Krupinsky, J.M., Bailey, K.L., McMullen, M.P., Gossen, B.D. and Turkington, T.K. 2001. Managing plant disease risk with diversified cropping systems. Agron. J. (In press)

c) **Other Scientific Publications**

1) Buchwaldt, L. and Gossen, B. 1997. Race identification in ascochyta blight of chickpea and anthracnose of lentil: A new initiative. Pg. 67 in A. Slinkard (ed.) Pulse Crop Research, Vol 2. Crop Development Centre, Saskatoon, SK. 111 pp.
2) Buchwaldt, L., Gossen, B. and Kutcher, R. 1997. Decision support system for fungicide application in pulse crops. Pg. 66 in A. Slinkard (ed.) Pulse Crop Research, Vol 2. Crop Development Centre, Saskatoon, SK. 111 pp.
3) Gossen, B.D. 1997. Fungicides reduce ascochyta blight severity in chickpea, 1996 and 1997. Report #76 in 1997 Pestic. Manage. Res. Rep., AAFC, Ottawa, ON, published electronically.

4) Gossen B.D., Kutcher, H.R., Bailey, K.L., Thomas, A.G. 1997. Effect of foliar application of azoxystrobin on diseases of pea, canola, wheat and barley in 1997. Report #101 in 1997 Pestic. Manage. Res. Rep., AAFC, Ottawa, ON, published electronically.
5) Hwang, S.F., Turnbull, G.D., Deneka, B.A., Gorda, M.V., Chang, K.F. and Howard, R.J. 1997. Effect of Quadris and Bravo sprays for control of Mycosphaerella blight of field pea. Report #79 in 1997 Pestic. Manage. Res. Rep., AAFC, Ottawa, ON, published electronically.
6) Kutcher, H.R. and Kirkham, C. 1997. Fungicide control of mycosphaerella blight of field pea at Melfort, Saskatchewan, in 1997. Report #79 in 1997 Pestic. Manage. Res. Rep., AAFC, Ottawa, ON, published electronically.
7) Kutcher, R. and Gossen, B.D. 1998. Pea disease control trial. Pg. 8-9 in Field Report 1997, Saskatchewan Conservation Learning Centre, Prince Albert, SK
8) Slinkard, A.E., Vandenberg, A., Warkentin, T.D., Xue, A., Hogg, T., Gossen, B., Kutcher, R. Hwang, S.F., and Blade, S. 1997. Methods of rating pea genotypes for reaction to mycosphaerella blight. Pg. 71-76 in A. Slinkard (ed.) Pulse Crop Research, Vol 2. Crop Development Centre, Saskatoon, SK. 111 pp.
9) Turnbull, G.D., Hwang, S.F., Deneka, B.A., Gorda, M.V., Chang, K.F. and Howard, R.J. 1997 Effect of scheduled sprays of Bravo on mycosphaerella blight of field pea. Report #81 in 1997 Pestic. Manage. Res. Rep., AAFC, Ottawa, ON, published electronically.
10) Buchwaldt, L., Chongo, G. and Gossen, B.D. 1998. Creation of a pulse disease web site. Pg 97 in Pulse Crops Research: Progress reports on pulse research in western Canada. Vol 3. 115 pp.
11) Buchwaldt, L., Gossen, B.D., Hwang, S.F., Kutcher, R., Turkington, K., Xue, A. 1998. Foliar fungicide reduces mycosphaerella blight severity in pea. Report #85. Pg 268-271 in 1998 Pestic. Manage. Res. Rep., AAFC, Ottawa, ON. (published electronically).
12) Buchwaldt, L., Gossen, B.D., and Chongo, G. 1998. Effect of foliar fungicide application on anthracnose severity in lentil, 1998. Report #91. Pg. 283-284 in 1998 Pestic. Manage. Res. Rep., AAFC, Ottawa, ON. (published electronically).
13) Gossen, B.D. and Vandenberg, A. 1998. Pulse pathology research: Who's driving and where are we going?. Pg 98-99 in Pulse Crops Research: Progress reports on pulse research in western Canada. Vol 3. 115 pp.
14) Anderson, K. L., Buchwaldt, L. and Gossen, B. D. 1999. Diseases of lentil in Saskatchewan 1998. Can. Plant Dis. Surv. 79: 126-127, published electronically.
15) Buchwaldt, L., Gossen, B.D., and Chongo, G. 1999. Effect of fungicide application for control of diseases in lentil 1999. Pg. 278-279, Report #100 In 1999 Pestic. Manage. Res. Rep., AAFC, Ottawa, ON. (published electronically).
16) Buchwaldt, L., Gossen, B.D., Hwang, S.F., Kutcher, R., and Chongo, G. 1999. Effect of fungicide application on control of mycosphaerella blight in pea 1999. Pg. 288-290, Report #105 In 1999 Pestic. Manage. Res. Rep., AAFC, Ottawa, ON. (published electronically).
17) Chongo, G. and Gossen, B.D. 1999. Ascochyta blight of chickpea in Saskatchewan in 1998. Can. Plant Dis. Surv. 79: 103-104. (published electronically).
18) Chongo, G., Gossen, B.D., and Buchwaldt, L. 1999. Fungicidal control of ascochyta blight in chickpea in 1999. Pg. 275-276, Report #99 In 1999 Pestic. Manage. Res. Rep., AAFC, Ottawa, ON. (published electronically).
19) Chongo, G., Gossen, B.D., Buchwaldt, L. and Lafond, G. 1999. Effect of Bravo and Quadris

on ascochyta blight and yield of chickpea in 1998. Pg. 272-274, Report #98 In 1999 Pestic. Manage. Res. Rep., AAFC, Ottawa, ON. (published electronically).
20) Anderson, K.L., Buchwaldt, L., Chongo, G., Gossen, B.D., Morrall, R.A.A., and Pearse, P. 2000. Diseases of lentil in Saskatchewan 1999. Can. Plant Dis. Surv. 80: 96-97.
21) Chongo, G., Buchwaldt, L., Anderson, K. and Gossen, B.D. 2000. Saskatchewan chickpea disease survey - 1999. Can. Plant Dis. Surv. 80: 86-87.

e) Proceedings and Abstracts

1) Gossen, B.D. and Rude, S.V. 1997. Incidence of *Mycosphaerella pinodes* in field pea samples, 1995-96. Can. J. Plant Pathol. 19: 327.
2) Chongo, G., Buchwaldt, L. and Gossen, B.D. 1998. Management of lentil anthracnose. Proc. Special Crops Conf., Edmonton AB, November 2-3, 1998.
3) Anderson, K., Bailey, K., Gossen, B., and Lafond, G. 1999. Impact of crop management system on disease severity of wheat and field pea 1995-1997. Can. J. Plant Pathol. 21: 193.
4) Armstrong, C.L., Gossen, B.D. and Chongo, G. 1999. Investigations of *Ascochyta rabiei* teleomorph occurrence in Saskatchewan. Can. J. Plant Pathol. 21: 193.
5) Buchwaldt, L., Anderson, K. and Gossen, B.D. 1999. Evidence for races of *Colletotrichum truncatum* on lentil (*Lens culinaris*). Can. J. Plant Pathol. 21: 199.
6) Chongo, G., Armstrong, C.L., and Gossen, B.D. 1999. Screening Canadian isolates of *Ascochyta rabiei* for mating types, teleomorph development and pathogenicity of ascospores. Can. J. Plant Pathol. 21: 199.
7) Chongo, G., Gossen, B.D., Kutcher, R., Gilbert, J., Turkington, K., Fernandez, M., and McLaren, D. 1999. Seedling susceptibility of roots of crop species to a foliar isolate of *Fusarium graminearum* of wheat. Pg.110 in Proc. Canadian Workshop on Fusarium Head Blight. November 27-30, 1999. Winnipeg, MB.
8) Hwang, S.F., Gossen, B.D., Turnbull, G.D., Chang, K.F., and Howard, R.J. 1999. Effect of seeding date and fungicidal seed treatments on root rot, seedling emergence and yield of lentil. Phytopathology 89: 35 (Supplem.).
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(C) Personnel

	FTE	Cost to Project
Professional		
- B.D. Gossen	0.3 PY	-
- R. Kutcher (AAFC Melfort)	0.1	-
- K. Turkington (AAFC Lacombe)	0.1	-
- S.F. Hwang (ARC, Vegreville)	0.1	-
- L. Buchwaldt	1.0	100%
- G. Chongo	1.0	100%
Technical		
- K. Bassendowski	0.4 PY	-
- Technician, Melfort	0.6	50%
Summer and Casual		
- four students per year	0.5	100%
Graduate Students		
- K. Anderson	0.5	100%
- C.l Armstrong	1.0	-

Recruitment

- Dr. L. Buchwaldt was hired in March 1998 as a research scientist on a service contract with the Saskatchewan Pulse Crop Growers Assoc. She was appointed as a research scientist with AAFC in July 1999, and promoted in April 2000 from RES 01 to RES 02.
- Dr. G. Chongo was hired as a NSERC Post-Doctoral Fellow in April 1998. His contract was renewed in 1999 and again in 2000.
- Krista Armstrong (M.Sc. student) joined the program in May 1998, but has also provided technical assistance for portions of the study.
- Cheryl Armstrong (M. Sc. student) joined the program in May 1998. She was initially supported from the grant, but was awarded a NSERC scholarship for 1999 & 2000.
- Four summer students were hired from May to August each year They worked on a part-time basis as required through the winter.
- Term technical assistants were hired as required

(d) Equipment - a bench-top incubator and purchased two desk-top computers for use by the professional staff and graduate students.

(e) Photos - available on the website.

(f) Innovation Fund Acknowledgement - AFIF funding has been acknowledged in all publications and presentations, and the AFIF logo appears on the front page of the "Pulse

Crop Diseases" web site.

(g) Public Information Materials

See listing under tech transfer and new technologies

(I) Deliverables for evaluation - N/A

Effect of plant age on resistance to *Ascochyta rabiei* in chickpea

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Accepted xxx

Short title: Plant age affects blight resistance in chickpea

Chongo, G. and Gossen, B.D. 200x. **Effect of plant age on resistance to *Ascochyta rabiei* in chickpea.** Can. J. Plant Pathol. xx: xxx-xxx.

The impact of plant age on resistance to ascochyta blight of chickpea (*Cicer arietinum*) was assessed on kabuli cvs. UC27 (susceptible), Sanford and B90 (partially-resistant) and desi cv. Myles (partially-resistant) using two aggressive isolates of *Ascochyta rabiei*. Tests were conducted under controlled conditions (repeated), and in field trials in 1999 and 2000. Seed was sown at regular intervals to produce plants at different growth stages (seedling, vegetative, flowering, podding), which were all inoculated at the same time in randomized complete block experiments with four replications . UC27 was highly susceptible at all stages of plant development. However, plant age had a large impact on the disease reaction of the partially-resistant cultivars. Two weeks after inoculation, blight severity in growth chamber studies was low in seedlings of these resistant cultivars (range among cultivars of 3-16%) and highest in podding plants (37-69%). Severity at 2-wk post-inoculation showed a similar pattern in the field trials, with mean severity among cultivars ranging from 2-3% in seedlings and 3-32% in podding plants. Severity at 8-wk post-inoculation on seedlings of the resistant cultivars was low (4-14% in 1999, 8-28% in 2000) relative to that in podded plants (56-88% in 1999; 45-85% in 2000) in the field trials. These results indicate that resistance decreases with plant age in the partially-resistant chickpea cultivars adapted to western Canada and that resistance alone may not provide adequate disease control.

Key words: ascochyta blight, epidemiology, disease management, *Cicer arietinum*, resistance

Introduction

Chickpea (*Cicer arietinum* L.) hectarage has expanded from only 800 ha in western Canada in 1995 to140,000 ha in 1999 and 280,000 ha in 2000 (Statistics Canada). Chickpea is a high-value, drought-tolerant crop that is adapted to the drier regions of the Canadian prairies. Including chickpea in traditional wheat-fallow cropping systems expands rotation options, and provides increased crop diversity with access to new export markets. However, chickpea production can be severely reduced by ascochyta blight [*Ascochyta rabiei* (Pass) Labrousse, teleomorph, *Didymella rabiei* (Kovacheviski) v. Arx.] (Chongo et al. 2000a). As a result, production is almost exclusively devoted to chickpea cultivars with the best resistance available (partial-resistance), even though they are generally not as well-adapted as certain susceptible cultivars . In 1999, yield losses of up to 96% (Chongo et al. 2000b) were observed when cool, wet weather persisted for long periods late in the growing season. Under these conditions, even resistant cultivars become heavily infected (Chongo et al. 2000b, Nene and Reddy 1987), and foliar application of fungicide during flowering reduces epidemic development and increases yield (Chongo et al. 2000b).

Ascochyta blight is characterized by tan-coloured necrotic lesions with dark margins that develop on all above-ground plant parts at every stage of plant development. Pycnidia develop within lesions, often forming concentric rings around the infection site (Nene and Reddy 1987). Infected stems are quickly girdled, killing all of the tissue above the lesion. Under favourable environmental conditions, blight can spread very quickly and cause substantial losses in seed yield and quality. Moderate temperatures (20°C) are optimal for disease development (Hafiz 1952, Trapero-Casas and Kaiser 1992a). Ascochyta blight can also spread quickly among fields

in a region due to dispersal of air-borne ascospores produced on overwintered plant residue (Armstrong et al. 2000, Trapero-Casas and Kaiser 1992b).

In two previous studies, plant age had no impact on disease reaction (Sattar 1933, Trapero-Casas and Kaiser 1992a), but other studies report that resistance declines as plants mature (Hafiz 1952, Nene and Reddy 1987). The significance of this difference among the studies is quite large; if resistant lines become susceptible to blight late in the growing season, it reduces the reliability of genetic resistance as the main strategy for managing this disease. The objective of these experiments was to investigate the impact of plant age on ascochyta blight severity in several chickpea cultivars that are grown commercially in western Canada.

Materials and methods

Inoculum preparation

Two aggressive, single-spore isolates of *A. rabiei* (Jan 9702 and Jul 9808) obtained from infected chickpea in Saskatchewan were used for all of the tests. Each isolate was grown in 10-cm diameter petri dishes on potato dextrose agar for 7 days on a laboratory bench under fluorescent light. The cultures were flooded with sterile water and conidia were dislodged using a sterile glass slide. For the growth chamber trials, he resulting spore suspensions were counted with a haemocytometer and diluted to 2×10^5 conidia mL^{-1}. For the field trial, rye seed was soaked in water for 72 h (48???), autoclaved, cooled and then sprayed with a conidial suspension of a single isolate. Containers of the inoculated grain were incubated at room temperature for 7 days, then air-dried.

Growth chamber trial

Three kabuli cultivars; UC27 (susceptible), Sanford and B90 (partially-resistant), and one desi cv. Myles (partially-resistant) were used in the trial. These cultivars were developed in the USA, but are grown commercially in western Canada. In the controlled environment trials, seedlings were grown in 13-cm diameter plastic pots filled with a soil-less mix of peat moss, vermiculite and sand (2:2:1 v/v/v) and a slow-release granular fertilizer (Osmocote, Scotts-Sierra Horticultural Products Co., Marysville, OH), with five plants per pot.

Seed was planted at regular intervals, to provide plants at all four major growth stages (seedling, vegetative, flowering and podding) for use in the study. UC27 and Myles normally flower about 7 days earlier than Sanford and B90. Therefore, seeding dates were adjusted to produce plants of each cultivar at the same stage of growth when inoculated, i.e. 2, 4, 6, 8 wk after seeding for UC27 and Myles; 3, 5, 7, 9 wk after seeding for B90 and Sanford. The seedlings were grown in a greenhouse at about 20/16°C (day/night) temperature, with 16 h photoperiod and 200-250 $\mu E\ m^{-2}\ s^{-1}$ light intensity from 430W high pressure sodium incandescent bulbs (Sonagro, Philips Co. USA). Plants were inoculated by spraying to runoff with a spore suspension of 2×10^5 conidia mL^{-1}, then incubated in a clear plastic mist chamber for 48 h at about 100% relative humidity, 20/16°C (day/night), 16 h photoperiod and 200-250 $\mu E\ m^{-2}\ s^{-1}$ light intensity supplied by cool white fluorescent and incandescent bulbs. After incubation, the pots of plants were arranged on a growth chamber bench in a randomized complete block design with 4 replicates.

Ascochyta blight severity was rated 2 wk after inoculation using the Horsfall-Barratt scale (Horsfall and Barratt 1945). The rating for each plant was converted to percent disease severity. At each plant age, observations on the time from inoculation to first appearance of symptoms

(latent period) were made each day and the number of lesions on the main stem were counted at 2-wk post-inoculation. As well, lesions on the top half of each stem were counted in podding plants. Each experiment was repeated once. The data was checked for homogeneity of variance using Bartlett's test, and then combined over the two repetitions in analysis of variance.

Field trial

Field trials were conducted on a heavy clay soil at the Agriculture and Agri-Food Canada Research Farm at Saskatoon in 1999 and 2000. The plots were 1.2 x 3m, with 0.3m row spacing, arranged in a randomized complete block design with 4 replicates. All four cultivars were used to assess the influence of plant age on resistance. Seeding rates were 95 g/plot for cv. UC27, 85 g for Sanford, 40 for Myles and 53 for B-90, to produce a plant density of about 55 plants m^{-2}. In 1999, seeding was delayed due to saturated soils, so the first seeding date was 26 May for Sanford and B-90 and 03 June for UC27 and Myles. In 2000 the first seeding date was 04 May for Sanford and B-90 and 08 May for UC27 and Myles. Thereafter, seeding was done every 2 wk for each cultivar for 8 wk, to provide plants at four distinct growth stages (seedling, vegetative, flowering and podding). Plots were inoculated by distributing equal volumes of rye-grain inoculum in each plot. In 2000, infested chickpea stubble was also applied late in the season to increase the disease pressure.

Ascochyta blight severity was rated and analyzed as described previously for the growth chamber studies. In the field trials, severity was assessed at 2, 4, 6 and 8 wk after inoculation. Each rating date in each year was analyzed separately using analysis of variance.

Results

Growth chamber trial

In the controlled environment study, plant age, cultivar and cultivar x age interactions had significant effects on blight severity on leaves and the number of lesions on stems, but only the main effects of cultivar and plant age were significant for stems (Table 1). Symptoms appeared 1-4 days earlier on UC27 than on the resistant cultivars (Table 2). On Sanford and B-90, the latent period decreased as plants matured (Table 2). On UC27, severity was not affected by plant age, but the number of lesions per stem increased as plants matured (Table 2). Lesions were evenly distributed on stems of UC27, but most lesions were concentrated on the bottom half of stems of the resistant cultivars (Table 2).

Infection frequency on the resistant cultivars increased substantially as the plants matured (Fig. 1, Table 2). For each plant part, the severity of ascochyta blight on seedlings of UC27 was as high as that on mature plants (Fig. 1). However in resistant cultivars, severity was low in seedlings (3% in Sanford, 2% in B90, 16% in Myles), but severity increased as the plants reached the podding stage (69% in Sanford, 37% in B90, 52% in Myles). Severity was lower on leaves than on stems, especially from flowering to podding (Fig.1). At podding, severity was lowest in B90, regardless of plant part. There was no difference in infection between the two isolates, so only the results for isolate Jan9702 are presented.

Field trial

The main effects of cultivar and plant age were significant ($P \leq 0.01$) for disease severity at each rating date, and there was a significant interaction between cultivar and plant age for 3 of 4 dates

in each year (Table 3). There was no effect of isolate in either year, so the results were combined across isolate for presentation? In 1999, there was no difference in severity among plant ages for UC27 on the first rating date (range 82-85%) but severity in the resistant cultivars ranged from 2-16% in Sanford, 2-6% in Myles and 1-3% in B90 (Fig. 2A). For the resistant cultivars, the most severely affected plants were those inoculated at podding. A similar trend was observed at the second, third and fourth ratings. At each rating date, severity was higher than at the previous date (Fig. 2B-D). At the fourth rating, severity in the oldest plants was 88 % in Sanford, 59% in B90, and 56% in Myles (Fig. 2D). The same pattern of disease increase with plant age and time occurred in 2000 (Fig. 3A-D). Blight severity was 2-3% in seedlings and 4-32% in podding plants of resistant cultivars two weeks after inoculation. By the fourth rating date, severity had increased to 8-28% in seedlings and 47-85% in podding plants (Fig. 3A & D).

Discussion

The development of ascochyta blight of chickpea was affected by plant age and cultivar. These factors influenced the onset of symptoms, infection frequency and disease severity.. Symptoms generally developed more quickly on the susceptible cultivar UC27 than on the resistant cultivars.

Results from the two repetitions of the controlled environment trial were supported by observations from both years of the field study. Plant age had little effect on disease progress in UC27, which was severely blighted regardless of growth stage. This supports the results of two studies that reported that plant age did not affect disease severity (Sattar 1933, Trapero-Casas and Kaiser 1992a). In contrast, the partially-resistant cultivars were highly resistant as seedlings, but

resistance declined sharply at flowering. This supports other studies that report increased susceptibility of chickpea as plants mature (Hafiz 1952, Nene and Reddy 1987). We conclude that the difference in results among these studies is likely due to the differential reaction of resistant and susceptible cultivars. Most lesions appeared to be concentrated on the bottom half of stems in the partially resistant cultivars, but were evenly distributed along the stems of the susceptible cultivar. This may suggest that plant part may have had an impact on expression of resistance or it may have simply been due to the increase in growth of new, healthy plant material at the top through stem lengthening.

In the field, there were substantial differences in disease reaction among the resistant cultivars. Blight severity was consistently lower in B90 and Myles than in Sanford. The controlled environment test showed a similar pattern, which suggests that the assessment protocol under controlled conditions can be used to predict blight response in the field. Cool and wet growing seasons in both 1999 and 2000 provided favourable conditions for development of ascochyta blight epidemics. Under these conditions, chickpea cultivars with partial resistance did not provide adequate disease control, and application of foliar fungicide may be required to maintain seed yield and quality (Chongo et al. 2000b, Shtienberg et al. 2000).

Based on the strong association between the disease reaction of cultivars across plant ages in the controlled environment and field studies, assessments of blight severity at two weeks after inoculation provide a quick and efficient way to assess the disease reaction of chickpea cultivars. However, blight severity on plants in the growth chamber study was higher at any stage of development than in the field trial. This is likely due to differences in environmental conditions, which are generally more variable in the field than under controlled conditions. This study also

shows that assessment of ascochyta blight on seedlings may tend to overestimate cultivar resistance.

We conclude that, in the partially-resistant cultivars adapted to western Canadian conditions, ascochyta blight resistance declines rapidly, starting at about the onset of flowering. To manage ascochyta blight in chickpea, growers should adopt an integrated package incorporating resistant cultivars, 4-year crop rotation, disease-free seed, and if necessary, fungicide application. Relying exclusively on cultivar resistance is unlikely to provide adequate disease control when conditions are favourable for disease development.

Acknowledgment: Thanks to the Agri-Food Innovation Fund for partial funding of the project, and to Ken Bassendowski and the summer student crew for technical assistance.

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Table 1. Analysis of variance of the effect of plant growth stage on ascochyta blight severity on four chickpea cultivars under controlled conditions.

Source	df	Leaves	Stems	Whole plant
Cultivar (C)	3	29692***	21662***	25230***
Plant age (A)	3	5192***	13370***	8632***
C x A	9	1184***	1922***	1418***
Error	105	165	175	118

*** significant at $P = 0.0001$

Table 2. Effect of plant age and cultivar on latent period and lesion number on chickpea inoculated with isolate Jan9702 of *Ascochyta rabiei* under controlled conditions.

Cultivar	Plant age (weeks)	Latent period (days)	Lesions/ stem	Lesions on top half of stem	
				number	%
UC27	2	5	7		
	4	5	10		
	6	5	15		
	8	5	26	0.08	54
Sanford	3	10	2		
	5	8	4		
	7	7	8		
	9	8	19	8 b	42
Myles	2	7	3		
	4	6	5		
	6	7	9		
	8	7	16	6 c	38
B-90	3	10	1		
	5	8	3		
	7	7	7		
	9	8	12	4 d	33
Cultivar		**	***		
Age		**	***		
Cultivar x age		**	ns		

, * significant at $P \leq 0.01$ and $P = 0.0001$, ns = not significant.

Means in a column followed by different letters are significantly different, $LSD_{0.05}$.

Table 3. Analysis of variance of the effect of plant growth stage on ascochyta blight severity (assessed at 2-wk intervals, post-inoculation) on four chickpea cultivars in a field trial at Saskatoon, SK in 1999 and 2000.

		1999 Mean squares				2000 Mean squares			
Source	df	Rating 1	Rating 2	Rating 3	Rating 4	Rating 1	Rating 2	Rating 3	Rating 4
Cultivar (C)	3	21014***	22808***	22581***	17777***	28157***	23230***	21720***	15324***
Age (A)	3	306**	3441***	4629***	5966***	321***	2784***	3605***	4195***
C x A	9	66	396**	758***	1058***	162**	358	397**	348**
Error	45	57	153	135	106	45	193	124	221

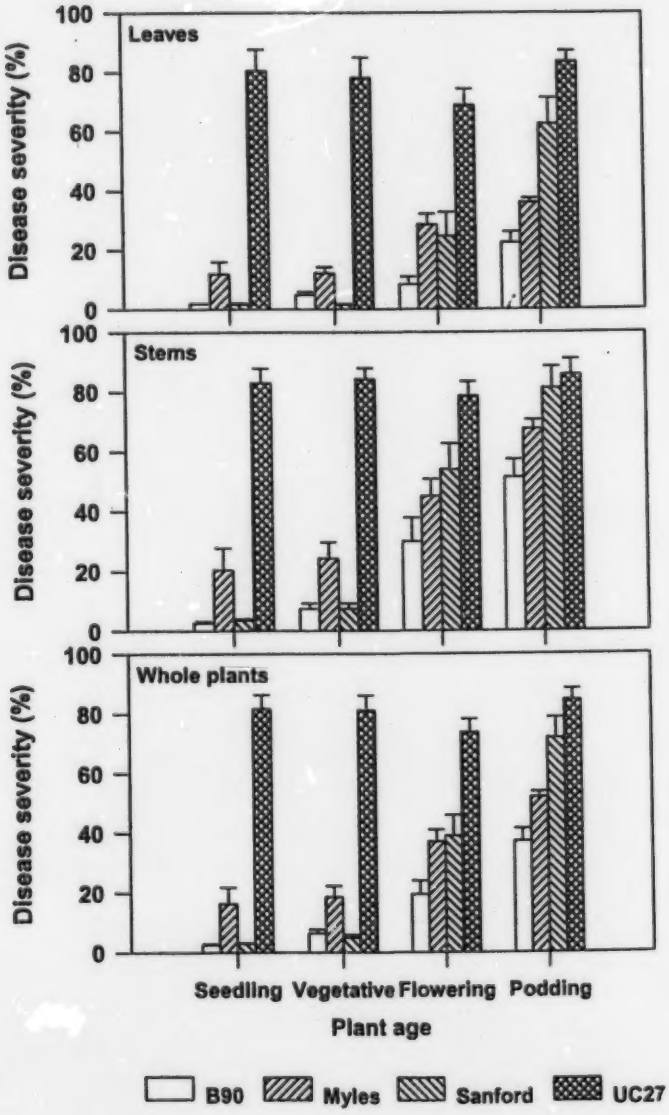
, * significant at $P = 0.01$ and $P = 0.001$, respectively

Fig.1. Ascochyta blight severity on leaves, stems and whole plants in four chickpea cultivars inoculated with isolate Jan9702 of *Ascochyta rabiei* at the seedling, vegetative, flowering and podding stages under controlled conditions.

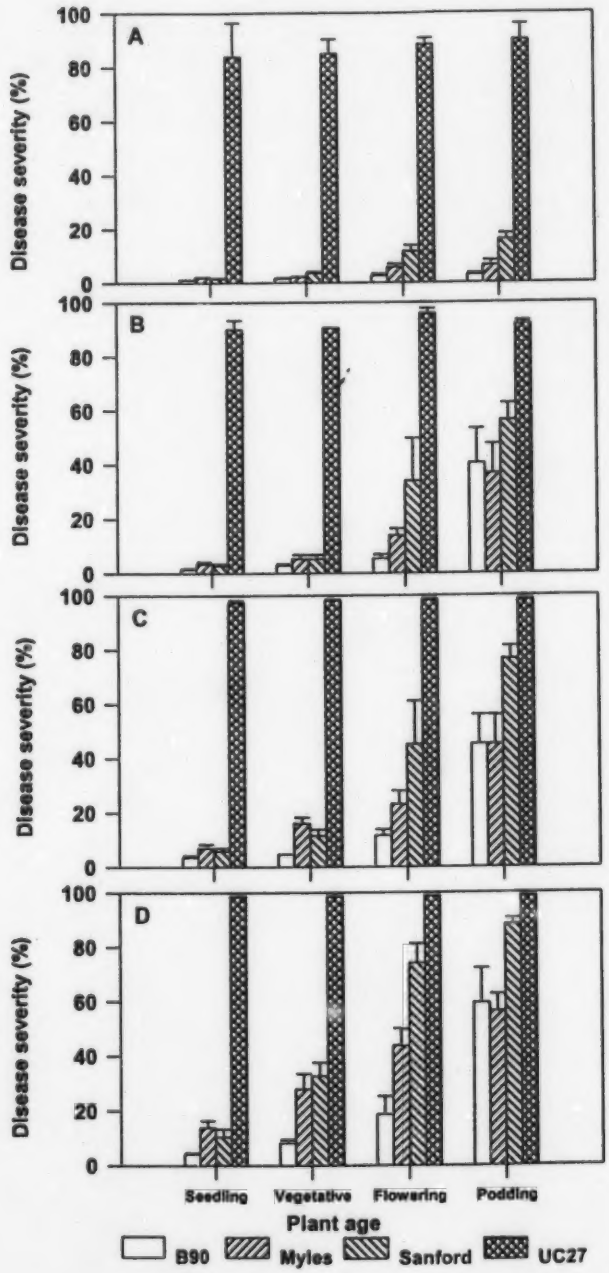
Fig. 2. Ascochyta blight severities in four chickpea cultivars inoculated with isolate Jan9702 of *Ascochyta rabiei* at different growth stages: **(A)** seedling, **(B)** vegetative, **(C)** flowering, and **(D)** podding stages and rated at 2, 4, 6, and 8 wk after inoculation, in a field trial at Saskatoon, SK in 1999.

Fig. 3. Ascochyta blight severities in four chickpea cultivars inoculated with isolate Jan9702 of *Ascochyta rabiei* at different growth stages: **(A)** seedling, **(B)** vegetative, **(C)** flowering, and **(D)** podding stages and rated at 2, 4, 6, and 8 wk after inoculation, at Saskatoon, SK in 2000.

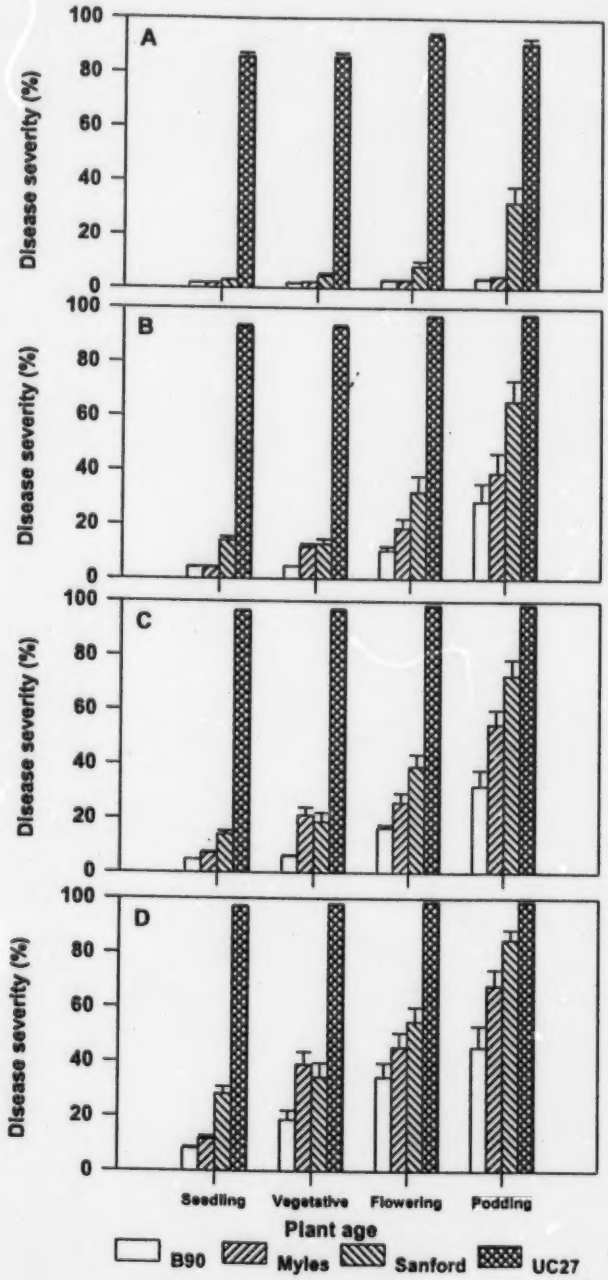
1 **Fig. 1.** Chongo



1 **Fig. 2.** Chongo



1 Fig. 3. Chongo



Bruce Gossen
Appendix 4

Screening of lentil for resistance to *Colletotrichum truncatum* and identification of two pathotypes

L. Buchwaldt, K. L. Anderson, R. A. A. Morrall, C. C. Bernier and B. D. Gossen

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ABSTRACT

L. Buchwaldt, K. Anderson, R. A. A. Morrall, C. C. Bernier and B. D. Gossen 200x. Screening of lentil for resistance to *Colletotrichum truncatum* and identification of two pathotypes. Phytopathology xx: xxx-xxx.

Lentil germplasm, a total of 1762 accessions, was screened for resistance to *Colletotrichum truncatum*, the cause of anthracnose. Most accessions were obtained from the World Lentil Collection, USDA-ARS (Pullman, Washington, USA), and 206 accessions were obtained from the Institut für Pflanzengenetik und Kulturpflanzenforschung (Gartersleben, Germany). All accessions were screened in the field by inoculation with a single virulent isolate. About 90% of the accessions were susceptible and not tested further. Retesting of 106 accessions with three isolates under controlled conditions resulted in identification of nine sources of anthracnose resistance, PI320937 (Germany), PI 345629 (Former Soviet Union), and PI468901 (Brazil) in the USDA collection, and Lens102, Lens104 (Germany), Lens106, Lens134 , Lens195 and Lens248 (Georgia), in the German collection. A quantitative and qualitative disease rating method was developed to distinguish between resistant and susceptible disease reactions. Heterogeneity occurred in the otherwise resistant lines, Lens104, 102 and 195, which had 7-13% moderately susceptible or susceptible plants, while the other accessions were uniformly resistant. Examination of pathogenic variability amongst isolates using seven resistant accessions demonstrated the presence of two different pathogenicity groups in *C. truncatum*. We assigned pathotype 1 to isolates of *C. truncatum* that were avirulent on the seven resistant accessions, PI320937, PI468901, PI345629, Indianhead, Lens104, 102, and 195; and pathotype 2 to isolates that were virulent on these accessions. About 40% of isolates collected in Saskatchewan and Manitoba belonged to pathotype 1, and 60% to pathotype 2. The frequency of the pathotypes isolated from plant material were almost the same, but pathotype 2 was isolated more frequently from seed. The presence of pathotype 2, for which as yet no resistance is identified, presents a risk to lentil production in western Canada.

In western Canada, more than half a million hectares have been planted to lentil annually in the past few years. Areas with intensive lentil production in Manitoba were devastated by widespread anthracnose in the nineties, when the disease was found in 40-50% of fields (Buchwaldt and Bernier, 1991; Buchwaldt et al., 1992), and contributed to a decline of the lentil acreage in this province. The disease spread to Saskatchewan and was identified in 30-60% of fields in 1998 and 1999 (Anderson et al., 1999; Anderson et al., 2000). Anthracnose is caused by *Colletotrichum truncatum* (Schwein.) Andrus and W. D. More. There are only a few reports worldwide of this disease. Lentil anthracnose haa been found in North Dakota (Venette et al., 1994), and Bulgaria (Kaiser et al, 1998), and a report from Ethiopia lists *C. destructivum* O'Gara on *Lens esculenta* (Stewart and Yirgou, 1967). Secondary sources have described the disease in both Pakistan and Brazil (Morrall, 1997).

In Canada, infested lentil residue is the primary source of inoculum, and the pathogen may survive up to four years when buried in the soil by tillage (Buchwaldt et al. 1996). Generally, when plants reaches the 10 to 12 node stage or early flowering, tan-coloured, necrotic lesions develop on the lower leaflets and a premature leaf drop occurs. Conidia, which are formed in acervuli, are dispersed to neighbouring plants by rain splash. Most damage occurs on the stems, which become covered with brown, necrotic lesions, and as the fungus penetrates the vascular tissue, the stems are girdling and turn blackish brown. After flowering, large patches of wilted and dying plants are evident in the field. Yield losses up to 70% have been reported (Chongo et al., 1999). Good control can be achieved with foliar applications of the fungicide chlorothalonil, but anthracnose resistant cultivars would be a better economical and environmentally sound control measure.

A preliminary screening of Canadian cultivars showed that all, except Indianhead, were highly susceptible to anthracnose (Gibson 1994). However, Indianhead has small, black seeds and is only suitable as a green-manure crop. The objectives of the present study were to identify other sources of resistance to *C. truncatum* by screening lentil accessions from germplasm collections, and to examine pathogen variability using identified sources of resistance as host-differentials.

MATERIAL AND METHODS

***Colletotrichum-truncatum* isolates.** Between 1991 and 1999, isolates of *C. truncatum* were obtained from anthracnose-infected lentil plants collected from commercial fields in Manitoba and Saskatchewan, and from two seed-testing laboratories. Stem pieces with anthracnose lesions were surface sterilized in 0.5% NaOCl for two minutes and rinsed in sterile water. Sections, 3-10 mm long, were plated either OMA (oat meal agar) amended with streptomycin (0.0139 g ml^{-1}) or SYAS (sodium-chloride yeast agar, sucrose) (Manandhar et al., 1986). amended with both streptomycin and chlortetracycline (0.0052 g ml^{-1}). Both media promoted conidial production. *C. truncatum* was also obtained from seed that had been surface sterilized in 2-3% NaOCl for 2-5 minutes, and plated on potato dextrose agar (PDA) for 7-10 days at room temperature. Dilute conidial suspensions were made from selected colonies, and 1.0 ml spread on 2.0% water agar (WA). After two days incubation, one or more germinating conidia were transferred to separate plates of OMA or SYAS. Isolates were stored for short periods as cultures on either SYAS or OMA at 5-7° C in darkness. Long term storage of isolates was either as infected, dry plant material at 5-7° C in darkness, or in at -80° C in a solution of 10% skim milk and 40% glycerol. Conidial suspensions for inoculation were prepared from 8-14 day old cultures incubated at room temperature under constant, cool-white, fluorescent light. Cultures were flooded with sterile water, and the surface scraped to dislodge conidia. Suspensions were filtered through Mira-Cloth (Calbiochem-Behring Corp., La Jolla, CA), adjusted to the desired conidial concentration using a hemacytometer (Hausser Scientific, 0.1 mm deep), and Tween 20 (polyoxyethylene-sorbitan monolaurate) at 20 µl L^{-1} was added as a surfactant.

Screening for anthracnose resistance. A total of 1556 accessions were obtained from the World Lentil Collection, USDA-ARS Regional Plant Introduction Station in Pullman, Washington, USA. The USDA collection consisted of about 2700 accessions of *Lens culinaris* subsp. *culinaris*. A large number of accessions were available from six countries, so only a part of these were screened for anthracnose (Bulgaria 46%, Chile 83%, India 25%, Iran 72%, Pakistan 39% Turkey 40%), while all accessions from the remainder 40 countries were screened (Table 1). A total of 206 lentil accessions from 23 countries were obtained from a collection at the Institut für Pflanzengenetik und Kulturpflanzenforschung in Gartersleben, Germany, 43 of these accessions were of unknown origin (Table 1).

Each year, 300-400 accessions were planted in small plots consisting of twelve accessions, and one row each of a susceptible cultivar, Eston, and the resistant cultivar, Indianhead. Forty seeds of each entry were planted in 0.5 m rows at 15 cm row spacing in 1.2m x 2.5m plots. Plants were inoculated 44-49 days after planting when the majority of accessions were at the10-12 node stage or early flowering. The soil surface was watered, and plants in each plot were mist-inoculated with 600 ml conidial suspension of a single virulent isolate of *C. truncatum*, JPPTNL882 at 4 x 10^4 conidia ml^{-1} using a 5.0 L hand-held sprayer. Plots were immediately covered with a sheet of polyethylene held by an A-shaped frame to maintain high humidity (Fig. 1A). To avoid high temperatures, inoculations were carried out in late afternoon, and the sheets were removed the following morning. Anthracnose symptoms developed on susceptible lines two weeks after inoculation. Amount of leaf and stem damage was rated as an averaged of all plants in a row 14-20 days and 29-36 days after inoculation (Fig. 1B). Accessions were scored on a scale with four categories, R = resistant, MR = moderately resistant, MS = moderately susceptible, and S = susceptible according to symptoms described in Table 2.

Accessions that were rated resistant (R) or moderately resistant (MR) in the field, were tested again under controlled conditions with more isolates. Each accession, including Eston and Indianhead, was planted in nine 9x9 cm pots (5-9 seeds per pot in a soil-less mixture, Metro mix, W. R. Grace & Co. Canada Ltd., Ajax, Ontario), and placed in a growth chamber at 20/16°C day/night temperature and 16 hr photoperiod. Three to four weeks after seeding, plants at the 10-12 nodes or early flower were inoculated with three isolates, JPPTNL882, 91IH and 91CHL, at 10^5 conidia ml^{-1} as described above. Three pots were used for each accession-isolate combination. Plants were incubated for 24 hr in a polyethylene tent maintained at 100% relative humidity achieved by a humidifier set at constant, cool-moisture production. A set of non-inoculated lentil differentials were included in each experiment as a negative control. Plants were transferred to a greenhouse with 12 hr supplement of high-pressure sodium light.

Preliminary tests showed that leaf damage was difficult to quantify, and further complicated by variable amounts of leaflets abscission. In contrast, stem infection could be quantified, and the appearance of lesions described as illustrated in Fig. 2. Lesions on the main stem were counted individually between 1 and 10, then in incremental steps, 15, 20, 25, 30 or >30 lesions. Depth of lesions was recorded as either superficial (spf) (Fig. 2A), deeply

B

penetrating (d) (Fig.2D, E), or a mixture (mix) (Fig. 2C) of superficial and penetrating lesions; the location of lesions was recorded as either on the stem-base (sb) (Fig. 2A), or the top half of the stem (t) (Fig. 2D, E); and finally, wilting was noted as either shoot die-back (sdb) (Fig.2 E), half of the plant wilted (½ w), or completely wilted (w). Six interaction phenotypes were observed using a combination of quantitative and qualitative factors (Table 3). Interaction phenotypes (IP) of individual plants were scored 11 to 15 days after inoculation, and the overall disease reaction of each accession-isolate combination was classified as either resistant (R), moderately resistant (MR), moderately susceptible (MS), or susceptible (S) according to Table 3.

Pathogen variability. Initially, four isolates, 91IH, 95M1, 95N32 and 95P40, were inoculated on fifteen lines with high levels of resistance identified in the above described screening of lentil germplasm. Seven accessions, which were resistant to one or more of the isolates, were used as a set of host-differentials to examine pathogenic variability of a larger population of *C. truncatum* isolates from western Canada. Forty eight single spore isolates from either plant material or seed were collected in Manitoba and Saskatchewan and cultured as described earlier. Six seeds of each accession were planted in 9x9 cm pots. As a control, seed of the susceptible cultivar Eston was either planted in six to eight separate pots in each experiment, or two seeds were planted in the centre of every pot together with an accession. Plants were grown in a growth cabinet at 20/16°C day/night temperature and 16 hr photoperiod (280-360 μ Eins m^{-2} sek^{-1}). After 21-24 days, when the plants had 10-12 nodes, they were inoculated with a conidial suspension of each isolate at 10^5 conidia ml^{-1} as described before. Each pot was wrapped in a 30 cm tall cylinder of polyethylene to support the plants, and prevent cross contamination amongst isolates. Plants were incubated for 24 hr and transferred to a greenhouse as described above. Fifteen experiments consisted of 5-7 isolates inoculated on the seven lentil differentials, but three experiments had 14-19 isolates. A set of non-inoculated differentials were included in each experiment as a negative control. Twenty three accession-isolate combinations were tested twice, 13 combinations were tested more than two times, and 12 combinations only once. The interaction phenotype of single plants were rated 11-15 days after inoculation as described above, the disease reaction for each accession-isolate combinations was determined after each experiment according to Table 3, as well as for two or more replications combined.

RESULTS

Characteristics of *C. truncatum*. Isolates were uniform with regards to spore morphology forming single-celled, hyaline and slightly falcate conidia, 15.6-25.0 x 3.5-4.0 µm, which were within the measurements (17-31.5 x 3-4.5 µm) defined by Tiffany and Gilman (1954), confirming that *C. truncatum* was the causal organism of lentil anthracnose in western Canada. Abundant conidia formed in salmon-coloured cultures on immersed, hyaline mycelium, with hyphal tips twisting anti-clockwise at the colony margin on both OMA and SYAS. Black setae and microsclerotia formed after 10-14 days.

Lentil accessions with resistance to *C. truncatum*. Over a five year period, 1762 lentil accessions from two lentil collections were screened for anthracnose resistance in the field by inoculation with a single isolate of *C. truncatum*. Each year, more than 90% of the accessions were eliminated as susceptible using a simple rating method of the amount of leaf and stem damage (Table 1). In the field, the resistant cultivar Indianhead developed few necrotic lesions on leaves, only superficial lesions on stems, and seed production was not greatly affected. More lesions were found at the bottom 4 cm of the stems, indicating that the stem base was more susceptible to infection than the upper half of the stems. In contrast, lesions on Eston, and other susceptible entries, were deeply penetrating occurring over the whole length of the stems, and gradually caused the plants to wilt (Fig. 1B). About 10% of accessions were rated resistant or moderately resistant in the field screening, and were retested under controlled conditions with three isolates. Isolate 91IH was more virulent than both 91CHL and JPPTNL882, but there were no specific interaction between the isolates and any of the lentil entries (data not shown). After retesting 106 accessions, nine accessions were identified with high level of anthracnose resistance. Three accessions, PI320937 (Germany), PI 345629 (former Soviet Union), PI468901 (Brazil) were from the USDA collection, and six accessions, Lens102, Lens104 (Germany), Lens106, Lens134, Lens195, and Lens248 (Georgia), were from the IPK collection in Germany (Table 4). All lines had small seed size, variable seed coat color, and either yellow or red cotyledon color. Four lines were from the European continent, one from Brazil, and four of unknown origin (Table 4).

Pathogenic variability of *C. truncatum*. The uninoculated set of lentil differentials did not show symptoms in any of the experiments. The susceptible control, Eston, was almost always severely infected when planted in separate pots, but served as an even better control when planted in the centre of the pot of each accession. A few experiments, or single pots, where Eston was not severely infected were excluded. Examination of pathogenic variability amongst 48 isolates of *C. truncatum* showed two distinctly different groups. One group (18 isolates) was avirulent on the seven lentil differentials but virulent on Eston (Fig. 3A), while the other group (30 isolates) was highly virulent on all these entries (Fig. 3B). In some experiments, a few accession-isolate combinations showed a reaction different from the two groups, but these results could not be reproduced. We assigned pathotype 1 to *C. truncatum* isolates that were avirulent on PI320937, PI468901, PI345629, Indianhead, Lens104, Lens102, and Lens195, and pathotype 2 to isolates that were virulent on these seven lentil entries.

Scoring of quantitative and qualitative symptoms on individual plants made it possible to study the frequency distribution of the six interaction phenotypes in each lentil differential. All plants of PI320937, PI468901, PI345629 and Indianhead were resistant or moderately resistant, while Lens104, Lens102 and Lens195 had 7-13% moderately susceptible or susceptible plants (Fig. 4). Segregation for resistance and susceptibility was especially evident in Lens195 which made it less suitable as a host differential (Table 5).

Of 48 isolates used in the present study, 60% were isolated from plant material and 40% from seed, 60% of the isolates were collected in Manitoba, and 40% in Saskatchewan (Table 5). Both pathotypes occurred in the two provinces at a frequency of 38% and 62% of pathotype 1 and 2, respectively. Of 11 isolations made from a single field in Neepawa, Manitoba, 7 isolates (no 5-11) belonged to pathotype 1 , and 4 isolates (no 26-29) to pathotype 2 (Table 5). Isolations made from plant material resulted in 45% of pathotype 1, and 55% of pathotype 2, while isolations made from seed resulted in 26% of pathotype 1, but 74% of pathotype 2 (Table 5).

DISCUSSION

Many lentil accessions, which appeared resistant to *C. truncatum* in the field, were in fact susceptible when retested under controlled conditions. Some entries likely escaped infection

because of uneven inoculation of field plots, drier conditions in outer rows, and variable pressure from secondary inoculum. Inoculation with the less virulent isolate JPPTNL882 at a lower concentration of 4×10^4 conidia ml^{-1} may also have contributed to lower disease severity in the field, compared to inoculation with the more virulent isolate 91IH at a higher concentration of 10^5 conidia ml^{-1}, when the entries were retested under controlled conditions. In addition to the cultivar Indianhead, nine new sources of anthracnose resistance were identified after screening 1762 lentil accessions. The three accessions, PI320930, PI468901 and PI345629, identified in the USDA World Lentil Collection, comprise 0.003% of *Lens culinaris* sbsp. *culinaris* that were screened (GRIN database). If accessions in the core collection alone had been screened, only PI 320937 would have been identified. The six accessions identified in the IPK collection represented 0.02% of the cultivated lentil entries (IPK data base). The low frequency of resistant accessions, particularly in the USDA collection, was likely due to the fact that the majority originated from Asia, where selection pressure from *C. truncatum* might has been absent. A narrow genetic diversity in cultivated lentil may also have contributed to the low incidence of disease resistant sources. Eastern Europe, including Germany, is probably a more likely region to identify anthracnose resistant germplasm, since some of the resistant lentil accessions originated within this large area. This is also supported by a publication from Bulgaria, where three cultivars, Stanka, Naslada and Stella, appeared to have had good anthracnose resistance in the field over several years (Mihov and Stoyanova, 1998).

In Canada, several crosses have been made with Indianhead to transfer anthracnose resistance into lentil of different marketable seed classes (Buchwaldt et al., 1995). The first anthracnose resistant cultivar, CDC Robin, was registered in 2000. Field tests showed that another line, 458-57, also derived from Indianhead, had 15%, 46% and 62% disease severity under low, medium and high anthracnose pressure, compared to 26%, 62% and 78% in Eston (Chongo et al., 1999). This demonstrated a positive effect of resistance to pathotype 1 under field conditions, where the natural population of *C. truncatum* is almost equally divided between pathotype 1 and 2, as shown in the present study. An examination of components of resistance expressed by PI 320937, PI 345629, Indianhead and 458-57, showed that fewer and smaller lesions were formed on stems and leaflets, and that incubation and latent periods were 24 and 32 days, respectively, compared to 11 and 13 days in the susceptible cultivar Eston (Chongo and

Bernier, 1999).

Two pathotypes were identified amongst isolates of *C. truncatum* from western Canada, but more pathogenic variation could potentially have been found using seven resistant lentil accessions originating from widely separated geographical regions. In the USDA data base (GRIN), accession PI468901 was described as highly susceptible in Brazil where it originated, indicating that pathotype 2, or a similar aggressive pathotype, was present there. Resistance to pathotype 1 was also found in eight other Brazilian lines, but these were heterozygous for cotyledon and seed coat color and were not pursued further. Pathogenic specialisation of *Colletotrichum* spp. has been demonstrated on many crop species. Several races of *C. lindemuthianum* on bean (*Phaseolus vulgaris*) have been identified (Menezes and Dianese, 1988), and two races of *C. trifolii* on alfalfa (*Medicago sativa*) have long been known and used in breeding programs (O'Neill, 1996). Specialisation has also been described in *C. orbiculare* of cucurbits (Wasilawa et al., 1993), and in *C. graminicola* of sorghum (Cardwell et al, 1989).

A genetic study of resistance genes in Indianhead, PI320937 and PI345629 is in progress. Preliminary results indicates that different genes in each accession confer resistance to pathotype 1. However, it is unlikely that combining of these genes in a single cultivar will confer resistance to pathotype 2. Thus, screening of wild lentil species, *L. culinaris* ssp. *orientalis, L. odemensis, L. nigricans* and *L. ervoides* for resistance to both pathotypes has been undertaken to identify resistance genes which could be transferred to cultivated lentil by interspecific crossing.

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Fig. 1. Field screening of lentil germplasm for resistance to anthracnose (A-B). A) Small plots (1.2 x 2.5 m) inoculated with a conidial suspension of *Colletotrichum truncatum* and covered over night with a sheet of polyethylene. B) Forty plots each of twelve lines, a resistant and a susceptible control three weeks after inoculation. Note the large number of plots with susceptible entries blackened by anthracnose compared to two green plots of the resistant line Indianhead.

Fig. 2. Illustration of some quantitative and qualitative symptoms of *Colletotrichum truncatum* on lentil stems (A-E). A) A few lesions on the stem base (sb). B) Superficial stem lesions (spf). B) A mixture (mix) of about 15 superficial and deep stem lesions. D) About 20 deep (d) stem lesions, some on the top (t) part of the stem. E) More than 30 deep (d) penetrating, girdling lesions and shoot die back (sdb).

Fig. 3. Disease reaction of four lentil accessions to two isolates of *Colletotrichum truncatum* (A-B). A) Isolate 95N32, virulent on the susceptible control Eston and avirulent on three lentil differentials Indianhead, PI345629 and PI 320937 (pathotype 1). B) Isolate 95S29 virulent on both the susceptible control and three lentil differentials (pathotype 2).

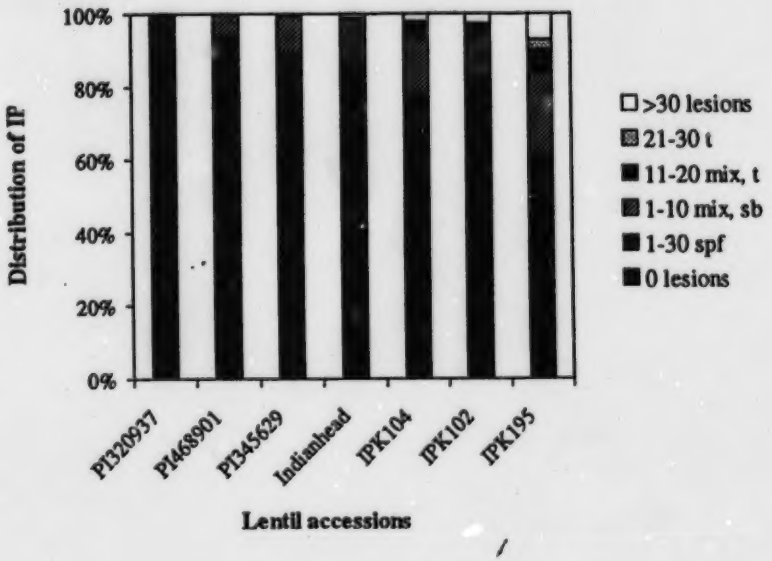

Fig. 4. Distribution of plants of seven lentil accessions by interaction phenotype (IP), 11-15 days after inoculation with nine isolates of *Colletotrichum truncatum* belonging to pathotype 1. Spf = superficial stem lesions, mix = a mixture of superficial and deep penetrating stem lesions, sb = lesions on stem base, t = lesions on top half of the stem.

Table 1. Number of lentil accessions which were screened for resistance to *Colletotrichum truncatum* (pathotype 1) obtained from the World Lentil Collection, USDA-ARS Regional Plant Introduction Station, Washington State University, Pullman, USA, and Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Gatersleben, Germany.

Country of origin	USDA Collection	IPK Collection
Algeria	1	
Afghanistan	10	7
Argentina	5	
Armenia	6	
Belgium	1	4
Brazil	13	
Bulgaria	91	
Canada	2	
Chile	167	1
China	1	
Croatia	1	
Costa Rica	1	
Cyprus	2	
Czechoslovakia	2	8
Egypt	15	1
England	1	
Ethiopia	7	3
France	6	1
Georgian		2
Germany	2	10
Greece	60	85
Guatemala	2	
Hungary	13	3
India	113	1
Iran	536	10
Iraq	20	2
Italy	8	10
Jordan	10	
Lebanon	19	1
Mexico	23	
Morocco	13	
Nepal	12	2
Pakistan	78	
Palestine	1	
Peru	3	
Portugal	1	
Romania		1
Russia	9	9
Soviet Union (former)	33	3
Spain	11	
Syria	12	
Tajikistan	3	1
Tunisia	1	
Turkey	190	
Ukraine	9	1
Unknown		43
USA	22	
Yemen	1	
Yugoslavia	19	1
Total	1556	206

Table 2. Scale for rating of anthracnose symptoms on lentil accessions in the field. One rating was given for the whole row 2-3 weeks and 4-5 weeks days after inoculation with a single virulent isolate of *Colletotrichum truncatum*.

Disease reaction	Anthracnose symptoms of lentil in the field
Resistant	Few leaf lesion, no stem lesions
Moderately resistant	Some leaf lesions and leaflet abscission, moderate number of superficial stem lesions
Moderately susceptible	Moderate defoliation, about half of the stem surface with deep penetrating lesions
Susceptible	Severe defoliation, more than half of the stem surface with deep penetrating lesions, which girdle the stems

Table 3. Interaction phenotypes for quantitative and qualitative rating of individual lentil plants 11-15 days after inoculation with *Colletotrichum truncatum*.

Disease reaction	Quantitative notation	Qualitative notation	Description of interaction phenotype (IP)
Resistant	0		No stem lesions
Moderately resistant	1-30	spf	Superficial stem lesions only (Fig. 2B)
	1-10	mix, sb	A mixture of superficial and deep lesions (Fig. 2C) Lesions on stem-base or lower half of stem (Fig. 2 A)
Moderately susceptible	15, 20	mix, t	A mixture of superficial and deep lesions Lesions of top half of the stem
	25, 30	t, sdb, 1/2w	Deep stem lesions including the top half of stem (Fig. 2D) Shoot die-back or plant half wilted
Susceptible	>30	sdb, 1/2w, w	Deep penetrating, girdling lesions on the whole stem (Fig. 2E) Shoot die-back, or plant half or completely wilted

Table 4. Lentil accessions with resistance to *Colletotrichum truncatum* (pathotype 1) identified in the World Lentil Collection, USDA-ARS Regional Plant Introduction Station, Washington State University, Pullman, USA, and in the collection at the Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany.

Accession	Other identifiers	Country of origin	Seed coat color	Cotyledon color
PI 320952	Indianhead[1]	Czechoslovakia	Black	Yellow
PI 320937	ILL505 (ICRISAT)	Germany	Black	Yellow
PI 345629	Novourenskaya 3565	former Soviet Union	Brown	Yellow
PI 468901		Brazil	Green	Yellow
Lens 102		Unknown	Black	Red
Lens 104	Schwarze Linse	Germany	Black	Red
Lens 106	Schwarze Linse	Unknown	Black	Yellow
Lens 134		Unknown	Black	Yellow
Lens 195	Okula	Unknown	Green	Yellow
Lens 248	B1156	Georgia	Grey	Red

[1] Indianhead served as a resistant control

No	No of reps	Isolate	Province	Source	PI 320937	PI 468901	PI 345629	Indianhead	Lens 104	Lens 102	Lens 195	Eston
1	4	91IH	MB	plant	R	R	R	R	R	SEG	R	S
2	1	JPPTNL882	MB	plant	R	R	MR	R	R	R	SEG	S
3	2	91B11	MB	plant	R	R	R	R	R	R	SEG	S
4	2	1a	SK	plant	R	R	R	R	MR	SEG	SEG	S
5	2	95N2	MB	plant	R	R	R	R	R	R	SEG	S
6	2	95N11	MB	plant	R	R	R	R	R	R	SEG	S
7	2	95N12	MB	plant	R	R	R	MR	SEG	R	SEG	S
8	2	95N20	MB	plant	R	R	R	R	R	R	SEG	S
9	1	95N21	MB	plant	R	R	R	R	R	R	R	S
10	1	95N23	MB	plant	R	R	R	R	R	R	R	S
11	4	95N32	MB	plant	R	R	R	R	R	R	R	S
12	4	95P40	MB	plant	R	R	R	R	R	R	R	S
13	2	92S12	MB	plant	R	R	R	R	R	R	SEG	S
14	1	9946-2	SK	seed	R	R	R	MR	R	MR	MR	S
15	1	8523-1	SK	seed	R	R	R	MR	MR	SEG	MR	S
16	1	72126	SK	seed	R	R	R	R	R	R	SEG	S
17	2	Apr9903	SK	seed	MR	R	MR	MR	R	R	R	S
18	2	Apr9904	SK	seed	R	R	R	R	MR	MR	SEG	S
19	3	95A8	MB	plant	S	S	S	S	S	S	S	S
20	3	95A10	MB	plant	S	S	S	S	S	S	S	S
21	2	95D1	MB	plant	S	S	S	S	S	S	S	S
22	3	95D35	MB	plant	S	S	S	S	S	S	S	S
23	5	95H14	MB	plant	S	S	S	S	S	S	SEG	S
24	2	95H15	MB	plant	S	S	S	S	S	S	SEG	S
25	4	95M1	MB	plant	MS	S	S	S	S	S	S	S
26	2	95N1	MB	plant	S	S	S	S	S	S	S	S
27	2	95N8	MB	plant	S	S	S	S	S	S	S	S
28	2	95N10	MB	plant	S	S	S	S	S	S	S	S
29	2	95N15	MB	plant	S	S	S	S	S	S	S	S
30	7	92R4	MB	plant	S	S	S	S	S	S	S	S
31	6	94R34	MB	plant	S	S	S	S	S	S	S	S
32	4	95S27	MB	plant	S	S	S	S	S	S	S	S
33	4	95S29	MB	plant	S	S	S	S	S	S	S	S
34	4	95SP31	MB	plant	S	S	S	S	S	S	S	S
35	1	May 9940	SK	seed	S	S	S	S	S	S	S	S
36	1	June 9903	SK	seed	S	S	S	S	S	S	S	S
37	1	1793	SK	seed	S	S	S	S	S	S	S	S
38	1	5696-1	SK	seed	S	S	S	S	S	S	S	S
39	1	9750-3	SK	seed	S	S	S	S	S	S	S	S
40	2	7864-1	SK	seed	S	S	S	S	S	S	S	S
41	2	7864-2	SK	seed	S	S	S	S	S	S	S	S
42	1	98G60	SK	seed	S	MS	S	S	S	S	S	S
43	2	Apr9908	SK	seed	S	S	S	S	S	S	S	S
44	2	Jun9903	SK	seed	S	S	S	S	S	S	S	S
45	2	May9908	SK	seed	S	S	S	S	S	S	S	S
46	2	Apr9920	SK	seed	S	S	S	S	S	S	S	S
47	2	Apr9921	SK	seed	S	S	S	S	S	S	S	S
48	2	Apr9905	SK	seed	S	S	S	S	S	S	S	S

R= resistant, MR= moderately resistant, MS=moderately susceptible, S=susceptible, SEG=lentil line segregating S/MR

Table 5. Disease reaction of lentil lines inoculated with single spore isolates of *Colletotrichum truncatum* isolated from plant material and seed collected in Manitoba (MB) or Saskatchewan (SK) demonstrating the presence of two pathotypes in western Canada. Pathotype 1, avirulent on seven lentil accessions, and pathotype 2 virulent on all accessions.

Appendix 8

Influences of *Pythium* spp. and effects of temperature, inoculum density, seed damage and metalaxyl seed treatment on seedling blight and seed yield of field pea

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Rough handling during harvest, cleaning, or seeding (augering or dropping) can damage field pea seed. Damaged seed is vulnerable to seed rot and damping-off caused by *Pythium* spp. When cool, wet conditions persist after planting, *Pythium* spp. can be an important constraint to stand establishment and yield in pea. Field plots were established to study the effect of pythium damping-off on seed yield of field pea and to assess the impact of seed damage on seedling establishment and fungicide efficacy. Seedling establishment and seed yield were enhanced by seed treatment with Apron fungicide and reduced by inoculation with *Pythium* and by mechanical damage to the seed. Fungicide seed treatment reduced the impact of seed damage, but did not restore seedling establishment and yield to the same level as undamaged seed. This study showed that planting fungicide-treated, high quality pea seed was the most effective means to maximize emergence and stand establishment in the field.

Introduction

Field pea (*Pisum sativum* L. var. *arvense* (L.) Poir.) cultivation has increased dramatically in the northern prairies of Canada in the past decade. The crop is well adapted to this region, where it is grown as a nitrogen-fixing cash crop that provides a break in cereal-based crop rotations. In the northern prairies, planting must occur between the last week in April and the first week of June. Frozen or saturated soils and risk of severe frost preclude earlier seeding, while later planting increases the risk of frost damage due to delayed maturity before harvest. Weather conditions during seeding and crop establishment are unpredictable. Air temperatures can drop by 30°C within a day, and snowfall can occur until late May. This slows germination and establishment of seedlings, and makes them more susceptible to infection by soilborne pathogens, especially *Pythium* spp. However, even in fields where the potential for losses due to pythium diseases is high, early seeding is required to maximize yield potential (Hwang et al., 1999), so most pea crops are planted as early as possible.

During seed germination and early establishment, seedling development takes place at the expense of reserves in the cotyledons. Most seed germination tests are conducted under optimal conditions; however, seedlings growing in cold soil develop more slowly and place greater demands on these reserves. Results of seed germination tests in the laboratory are often similar to those conducted under favourable conditions in the field, but tend to overestimate germination under adverse field conditions, especially at low soil temperatures (Wellington, 1962). As a result, seedlots with similar germination results under laboratory conditions may differ markedly in establishment under adverse field conditions (Duczmal and Minika, 1989).

Assessments of seedling vigour help determine the potential for rapid, uniform emergence and development of seedlings under a wide range of field conditions (AOSA, 1983). These tests

were developed to overcome the problems with laboratory tests for germination (Duczmal and Minicka, 1987). Lack of seedling vigour results in delayed emergence and weak plants. Moreover, slow emergence may delay critical growth stages and result in lower seed yield. Poor vigour may also lead to seedling mortality through increased susceptibility to disease (Schlub and Schmitthenner, 1978).

Mechanical damage to seed is one of the prime determinants of seedling vigor (Brand, 1999). Field pea is a large-seeded crop with a relatively thin seed coat, which makes the seed especially vulnerable to mechanical damage during harvesting, handling and planting. Mechanical damage results in increased uptake of water and increased leakage of solutes from seeds. Nutrient leakage not only reduces the nutrients available to the seedling, but also triggers the growth of soilborne pathogens such as *Pythium* spp. (Schroth and Cook, 1964). Nutrient-deficient seedlings also grow more slowly and have reduced resistance to infection by soil-borne pathogens because the damaged seeds have a greater surface area available for attack (Stasz *et al.*, 1980).

Pythium spp., which are ubiquitous in cultivated soils, play an important role in the root rot disease complex on pea, particularly in soils that are cold and poorly drained (Benedict, 1969; Kraft and Burke, 1971; Kraft and Roberts, 1969; Lumsden *et al.*, 1976, Hwang and Chang, 1989). In the northern prairies, pythium seed rot and seedling damping-off is a major factor limiting the production of field pea in some years (Hwang and Chang, 1989). With repeated cultivation of field pea, the incidence and severity of seedling and root diseases will rise as populations of *Pythium* spp. increase. Seedling diseases reduce stand uniformity i.e. both stand density and plant growth stage, which is required for maximum yield. Root diseases caused by *Pythium* spp. also reduce nodulation and nutrient utiliztion of the field pea crop, resulting in losses in yield and quality (Xi et

al., 1995).

In regions where pythium injury on peas is common, fungicide seed treatments are widely used. Metalaxyl [Apron®, Gustafson, Plano, Texas], one of the most commonly used fungicides, controls members of the Peronosporales by inhibiting RNA and protein synthesis (Fisher and Hayes, 1982). It is systemic and has activity against pythium seed decay and seedling blight, as well as root rot (Cook and Zhang, 1985, Hwang 1988; Hwang *et al.* 1995, 1999). The other option for growers is to use vigorous seed to speed emergence and shorten the period where seedlings are highly susceptible to infection by *Pythium* spp.

The objectives of this study were to: i) isolate and identify the species of *Pythium* associated with diseased field peas in Alberta and to determine their pathogenicity and relative virulence to field pea seedlings, ii) study the influences of inoculum density on the development of pythium root rot, iii) evaluate the effects of seed treatment with metalaxyl on seedling establishment and yield, iv) compare the effects of different formulations of metalaxyl on seedling establishment and yield, and v) examine the interacting effects on seed damage and fungicide seed treatments on improving seedling establishment and seed yield of field pea.

Materials and Methods

Growth conditions

All of the controlled-environment studies reported in this paper were conducted using 15 cm-diameter pots filled with a steam-pasteurised (121°C for 2 hr) mixture of loam, sand, and vermiculite (3:1:1, v/v). For all tests, seed was surface disinfested in 70% ethanol for 2 min, followed by 2 min in 0.6% NaOCl, then rinsed three times in distilled water. Seeds were planted 1.5-cm deep in each pot. Pots were randomly arranged in a controlled environment growth chamber

at 20°/15°C (day/night), with a 16-hr day. Light intensity of 300 $\mu Es^{-1}m^{-2}$ was provided by cool-white fluorescent tubes and incandescent bulbs. Plant height was measured from the soil surface to the top leaf. Shoot and root dry weights were determined after plants were dried at 70°C for 24 hr. Pots were watered as necessary with distilled water.

Isolation of *Pythium* species and pathogenicity studies

The *Pythium* spp. used in these studies were isolated from rootlets and necrotic root tips found on pea plants sampled from fields in central Alberta. The necrotic tissue was surface-sterilized and placed on *Pythium*-selective pimaricin-vancomycin agar medium (MPVM) with rose bengal at 0.01 g/L (Mircetich 1973). The MPVM dishes were incubated for 48 hr in darkness at 20°C. Hyphal tips of mycelia growing from root tissue were transferred onto cornmeal agar and the resulting representative fungal cultures were identified by National Fungal Identification Service, Centrao Experimental Farm, Ottawa, Ontario (according to the description of Middleton (1943)) and used in subsequent pathogenicity studies.

One colonized 6-mm-diameter agar disk, taken from 4-day-old cultures of each of the 26 different Pythium isolates, was grown on a 20% V8 juice agar plate for 10 days. The inoculum was prepared by comminuting fungal culture from a 90-mm petri plate with 200 ml of distilled water for 15 sec in a Waring Blendor: 25 ml of the inoculum of each of the 26 isolates was poured over pots containing a steam-pasteurized soil mixture. Noninoculated V8 juice agar culture was used as a control. After applying the inoculum, 5 cm of steam-pasteurized soil mixture was added on top of the inoculated soil. Ten seeds of field pea cv. Express were planted in each of ten replicate inoculated pots. Pots were randomized in a growth chamber, and the experiment was repeated using seeds of cv. Radley. Percent seedling survival and plant height

were recorded 2 weeks after seeding and shoot and root dry weights were determined one month after seeding.

Fungal inoculum

The cornmeal-sand inocula were produced according to the method described by Hwang (1988). For the controlled-environments studies of seed injury and seed treatment, cornmeal-sand inoculum of each isolate of *P. irregulare* (P-3 and P-13) and *P. ultimum* (P-29 and P-31) was produced according to the method described by Hwang (1988). The inoculum was then mixed with an autoclaved sand and loam mixture to the desired inoculum density.

For field studies of seed injury and seed treatment, grain inoculum was prepared by inoculating autoclaved grain (rye-oat mixture, 1:1 v/v) with the isolates used in the controlled environment studies. Inoculated jars were incubated for 2 wk at room temperature and shaken periodically to ensure complete colonization of the grain. After incubation, the infested grain was air-dried, ground and stored at 4°C until needed.

Effect of inoculum density

The five inoculum densities of isolate P-29 of *P. ultimum* were adjusted by adding the infested, cornmeal sand inoculum to steam-pasteurized soil mixture in ratios (w/w) of 1:50, 1:100, 1:200, 1:500 and 1:1000. These rates of inoculum provided population densities between 4×10^4 and 0.2×10^4 cfu/g of soil mixture. Pasteurized soil mixture that had received noninfested cornmeal sand inoculum (1:200, w/w) was used as the control. Soil mixture and inoculum were mixed in a twin-shell blender for 1 h, then immediately potted. The experiment employed a randomized complete block design with 10 replicate pots per each treatment and ten surface-

disinfested seeds of Express field pea were planted per pot. Percent stand counts were made at 8 intervals over 26 days after planting and results were expressed as percentages. Shoot and root dry weights per pot were recorded one month after seeding. The experiment was repeated using seed of cv. Radley, and since the results were nearly identical, the data were pooled to represent the average of the experiments.

Metalaxyl seed treatment

The inoculua containing six virulent Pythium isolates were mixed with a soil mixture to provide a density of 10^4 cfu/g soil mixture, respectively. A 6 x2 factorial design was used with separate pots filled with soil inoculated with each of six pathogenic *Pythium* isolates as one factor and two levels of seed treatments (with and without metalaxyl (Apron FL, Gustafson) at a rate of 0.35 ml a.i./kg seed) the other factor. Ten seeds of field pea cv. Express were planted in each pot, and six replicate pots were used for each treatment. Percent seedling survival, plant height and shoot and root dry weights were recorded as previously described. The experiment was repeated using seed of cv. Radley, and since the results were nearly identical, the data were pooled to represent the average of the experiments.

Effects of seed damage

Under controlled environment. Seed of the pea cv. Carneval was surface-sterilised and treated with metalaxyl as described above. The control seed did not receive any treatment. A cornmeal-sand inoculum of *P. irregulare* (P-3) was mixed thoroughly with the steam-pasteurized soil mixture at a rate of 500 cfu/g soil mixture. Seeds were planted 2 cm deep in 12 cm fibre pots. The four treatments were: i) control (vigorous seed, untreated), ii) vigorous seed + metalaxyl, iii) damaged

seed + metalaxyl, iv) damaged seed, untreated.

Pots were half filled with autoclaved soil, then mixtures of infested soil were added to fill each pot. Then 10 damaged or non-damaged seeds of pea cv. Carneval were planted at 3-cm depth. Noninoculated soil was used for the control. The experiment was evaluated at two air temperature regimes, 6/12°C and 10/20°C. The experiment was arranged in a completely randomized design, with 10 pots per treatment. Seedling survival and plant height were assessed as described previously. Root rot was evaluated before roots were dried. The experiment was conducted twice using *P. ultimum* (isolate P-29) as the inoculum source, but only one representative experiment was selected for presentation.

Under field conditions. A split-split-plot design with four replications was employed to establish plots at Vegreville, AB on 28 May 1996. The field pea cvs. Carneval and Montana were the main plots, inoculation with *Pythium*-infected grain vs. sterile grain were the sub-plots, and undamaged, auger-damaged and drop-damaged seeds were sown with and without Apron fungicide were the sub-sub plots. Auger damage was induced by 3 repeated cyles through a 1-metre on-farm treater auger (Gustafson OSP, Plano, TX). Drop damage was induced by storing seed overnight at -20° C then dropping 4 metres. Each plot consisted of 4 rows, each 6m long and spaced 25 cm apart. Seeds were planted 5 cm deep with a v-belt seeder at a rate of 50 seeds/row. Inoculum (40 ml/row) was planted with the seed. Emerged seedlings were counted on 5-m lengths of the two middle rows of each plot two weeks after seeding. Plots were harvested on 10 September 1996 and seed yield was determined. The experiment was repeated in 1997 at Brooks, AB, with seeding on 30 May and harvest on 27 August, 1997.

A smaller version of the trial was planted at Namao, AB in 1998 using Carneval and Profi

as the main plots and drop damaged vs. non-damaged seed in sub-plots. Seedlings were counted on 2-m lengths of the two middle rows two weeks after seeding plots were seeded on 25 May and harvested on 2 September.

Comparison of different Apron formulations

Field pea cvs. Carneval and Carrera were seeded on 14 May and 31 May, 1997 at Brooks and Vegreville, Alberta, respectively, in a split-plot, randomized complete block design with four replications. Pea cultivars served as main plots and fungicides as subplots. Each subplot consisted of four, 6 m rows of plants spaced 20 cm apart. Seeds of Carneval and Carrera were planted 5 cm deep at a rate of 22 g and 20 g per row, respectively. Seed was treated with APRON XLS (metalaxyl 360 g/L LS), APRON FL (metalaxyl 317 g/L SN) in a Hege II small batch seed treater at a rate of 0.5 and 1.1 ml/kg seed, respectively. Inoculum containing a mixture of *P. ultimum* and *P. irregulare* was incorporated at the time of seeding at the rate of 40 ml/row. Emerged seedlings were counted three weeks after seeding along 6 m of the two middle rows of each plot. Twenty plants were sampled from each plot and the roots were assessed for root rot severity (percentage root area discolored). At maturity, plots were harvested by small plot combine on 18 and 27 August, 1997, at Brooks and Vegreville, respectively. Seeds were weighed to determine yields.

Data analysis. Field data were assessed using analysis of variance and means were compared using Duncan's Multiple Range Test (SAS Inst. Inc., 1985). Bartlett's test was used to assess homogeneity of variances among sites. Analyses of variance among inoculum densities were performed on percentage damping-off for each pathogen or the two in combination. The data

were arcsine transformed before analysis to improve homogeneity of the variance. To compare the inoculum density for each isolate, a natural log (square root) transformation was applied to inoculum density values to linearize the relationship with percentage damping-off.

Results

Fungal isolation and Pathogenicity

Pythium irregulare, P. ultimum, and four asexual groups of *Pythium*, Group1, 2, 3 and 4 (Dick 1990; Martin 1990) were identified from 26 isolates obtained from field pea seedlings collected from commercial fields in Alberta. The virulence of these isolates on field pea cvs. Express and Radley is presented in Table 1. There were substantial differences in pathogenicity among isolates within *P. irregulare, P. ultimum*, and Groups 1 and 2. Within each taxonomic group, some isolates killed almost every seedling, while other isolates were only slightly pathogenic. The same trend for these isolates was subsequently observed in the height and root mass of the young plants. There were only 2 isolates of Groups 3 and 1 isolate of Group 4 in the test, and none of these isolates were highly pathogenic on field pea.

Effect of inoculum density

Root rot infection occurred at all inoculum densities of *P. ultimum*. The relationships between inoculum concentration of *P. ultimum* and percent seedling survival (Table 2) and the shoot and root dry weights were described by the linear model ($Y=a+bX$) and all R^2 values were significant at $P \leq 0.05$ (Figs. 1A-1B). Percent seedling survival and shoot and root dry weights decreased with an increase in inoculum density. Reductions in percent seedling survival and shoot dry weight were observed at 2×10^4 and 1×10^4 cfu/g for cvs. Express and Radley, respectively. The noninfested controls had the greatest seedling survival and the highest shoot and root dry weights. No root rot symptoms occurred in field pea plants grown in uninfested soil. The negative slopes of the regression lines for percent seedling survival and for shoot and root dry weights obtained for Express were greater ($P \leq 0.05$) than those of Radley (Figs. 1A and 1B).

Metalaxyl seed treatment

There was an interaction between *Pythium* isolate and seed treatment with metalaxyl for all of the variables assessed in the trial. The impact of the six *Pythium* isolates differed substantially in treatments without metalaxyl, including differential impact on seedling emergence and the height and weight of plants (Table 3). Where seed was treated with metalaxyl, there were no differences among isolates or between infested soil and the control.

Comparison of Apron formulations.

Significant effects ($P \leq 0.01$) occurred for cultivar, year and treatment for seedling emergence at both sites. Year*treatment interactions also occurred at both sites for seedling emergence. For seed yield, treatment effects occurred at both sites. There were differences between years at Vegreville, but not at Brooks, and differences between cultivars at Brooks, as well as an interaction between cultivar and year (Table 4). Both fungicide seed treatments improved ($P \leq 0.05$) seedling emergence and seed yield over the untreated controls. No differences in seedling emergence, root rot severity, or seed yield were observed between the two fungicidal seed treatments at either location (Table 5). Inoculation with the *P. ultimum* - *P. irregulare* mixture significantly reduced emergence in cv. Carrera and yield of cv. Carneval at both locations (data not shown).

Effect of seed damage

Under controlled environment.

Treatment gave a significant response in emergence, shoot and root dry weight and in root rot severity. Significant ($P \leq 0.01$) interactions occurred between treatment and temperature

for all parameters measured (Table 6). Temperature did not affect seedling emergence, but lower temperatures significantly ($P \leq 0.01$) reduced shoot and root productivity and exacerbated root rot severity. Nontreated pots had significantly lower emergence than those treated with metalaxyl. Damaged and nondamaged seed had similar levels of survival and shoot productivity where treated with metalaxyl, but survival and shoot and root productivity was significantly ($P \leq 0.05$) lower for untreated damaged seed and root rot severity was higher (Fig. 2).

Under field conditions. Variances were not homogeneous ($P \leq 0.01$) between sites for seedling emergence or seed yield. Therefore, analysis of variance was performed separately for each site (Table 7). Seed treatments with metalaxyl increased ($P \leq 0.01$) seedling emergence and yield at all three sites, except for seed yield in 1997. *Pythium* inoculation reduced ($P \leq 0.05$) seedling emergence and yield only in 1998 (Table 8).

There were differences between cultivars ($P \leq 0.01$) for seedling emergence and seed yield at all three sites, except for seed yield at Brooks, but there were no interaction effects in analysis of variance at any site (Table 7). Seed yield of Carneval was higher than Montana at Vegreville, equal to Montana at Brooks and higher than Profi at Namao (Table 8). Inoculation with *Pythium* reduced emergence and yield in Montana, but not in Carneval (data not shown), however, when combined across cultivars, inoculation reduced seedling emergence and seed yield only at Namao. In general, seedling emergence was highest where non-damaged seed was treated and lowest where seed was damaged and not treated.

In 1996 and 1997, damaged seed had lower seedling emergence than undamaged controls, when the same levels of seed treatment were compared. Seed yield was lower ($P \leq 0.05$) for all damaged seed in 1997 and 1998, but only for untreated, auger-damaged seed in

1996. Treatment with metalaxyl improved seedling emergence over untreated plots in all cases, but while seed yield was improved in all cases by metalaxyl in 1996, it remained at similar levels as for untreated seed in 1997 (Table 8). Healthy seed treated with Apron always produced higher yields than damaged seed without treatment.

The mean daily soil temperature at 5-cm depth for the first 10 days after planting at Vegreville ranged from 7.4° to 21°C, with a mean of 14.3°C). A shower (7 mm) occurred 2 days after planting and a significant rainfall (18 mm) occurred 1 week after planting. At Brooks, mean daily soil temperature ranged from 12.8° to 25.5°C with a mean of 18.1°C. Two minor showers (less than 5 mm) were recorded over those 10 days. At Namao, temperature ranged from 6.8° to 26°C with a mean of 17°C. Four minor showers occurred following planting.

Discussion

Pythium ultimum and *P. irregulare* are the principal causes of reduced seedling establishment due to seed rot and damping-off of field pea in the northern prairies (Hwang and Chang, 1989). This study identified six morphological types of *Pythium* from samples collected through a wide area of central Alberta. These varied widely both within and among types with regard to pathogenicity on field pea. Under controlled environment conditions, seed rot and seedling damping-off caused by these *Pythium* spp. were as severe in cool soils (10/20°C night/day regime) as in cold soils (6/12°C), but root rot was more severe in the cold soil. Previous studies have usually compared cool soils (10-20°C) with warm soils (> 20°C) and reported slower establishment and greater disease susceptibility in cool soils. However, the optimum temperature for pythium injury on garden pea seed and seedlings is 16 - 20°C, with moderate infection at 30°C, and lower infection at 4° than at 8°C (Leach 1947). Our study

indicates that the optimum temperature for pythium seed rot and damping-off of field pea is 15 - 20°C, while root rot is more severe at lower temperatures. Measurement of soil temperatures at 5 cm depth in our field trials confirmed that the 6/12°C regime approximates soil temperatures in the region early in May, while 10/20°C reflects temperatures in late May and early June.

In growth cabinet, greenhouse and field trials, inoculation with *Pythium* spp. reduced seedling emergence, but treating seed with the fungicide metalaxyl reversed this effect. In this study, metalaxyl provided useful levels of protection against root rot, allowed more nodule formation, and resulted in higher seed yield than an inoculated control. In controlled environments, metalaxyl more than doubled seedling emergence (Table 3). Under field conditions, most treatments increased seedling emergence by 10 - 40%. The same trend, with proportionally smaller differences, was noted in seed yield under field conditions, indicating that some compensation occurred for loss of plants early in the season (Table 5).

There was no significant difference in seedling emergence between healthy and damaged seed in an inoculated growth cabinet trial (Fig. 2), but mechanical damage resulted in reduced emergence in 2 of 3 field trials (Table 8). This confirms a previous report that *Pythium* caused increased incidence and severity of disease on damaged seed, especially at lower temperatures (Stasz and Harman, 1980). Leachates from damaged seeds promote rapid growth and colonization of the seed by *Pythium* spp. (Schlub and Schmitthenner 1978). Where the soil is low in nutrients, *Pythium* spp. may depend on exudates from damaged seed for germination and ultimately for successful host infection (Elad and Chet, 1987).

Clearly, damage caused by rough or repeated handling of seed can substantially reduce pea seedling establishment. Also, the impact is likely to be highest in soils that are heavily infested with *Pythium* spp. Our results support reports (Hull 1937) that pre-emergence rotting of

pea in cold wet soil can be a major cause of stand establishment problems. Even when mechanical seed damage did not affect emergence, the growth and productivity of plants grown from damaged seed was lower than that of plants from healthy seed (Tables 6 and 8). Therefore, only seed lots with good vigor should be used for early planting. Seed damage can be reduced by modifications to handling equipment, careful attention to equipment settings, and by controlling the moisture content of the seed at harvest.

Use of vigorous seed and/or treatment with metalaxyl can reduce the impact of pythium seed rot and damping-off on plant establishment and yield. High quality seed produces more rapid, uniform establishment in the highly variable conditions common early in the growing season and metalaxyl reduces infection of seedlings. It reduces damage to roots as seedlings become established, due to its systemic activity, and has a negligible effect on nodulation in pea roots (Hwang, 1988, 1994). Improved root rot disease control should contribute to increased yield and profitability of field pea and further increase its production by producers in the northern plains region

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Table 1. Pathogenicity of different *Pythium* isolates from field pea roots to pea cvs. Express and Radley seedlings[z]

Isolate	Survival (%)		Plant height (cm)		Shoot dry weight (g)		Root dry weight (g)	
	Express	Radley	Express	Radley	Express	Radley	Express	Radley
P. ultimum								
P-29	23	54	0.5	2.9	0.19	0.99	0.35	0.78
P-59	46	70	1.6	2.6	0.61	0.96	1.05	1.33
P-61	41	50	2.0	2.1	0.72	0.85	0.58	0.68
P-113	11	47	0.3	2.3	0.08	0.87	0.64	0.80
P-160	31	64	0.9	3.7	0.36	0.95	0.52	1.14
P-167	32	37	1.1	1.5	0.53	0.59	0.49	0.39
P-182	16	68	0.4	4.2	0.16	1.35	0.45	0.79
P. irregulare								
P-3	35	18	1.4	0.5	0.49	0.34	0.43	0.85
P-10	45	39	2.1	1.0	0.83	0.41	0.78	0.52
P-13	42	47	1.6	1.6	0.63	0.74	0.61	1.02
P-31	49	50	1.9	2.6	0.71	0.90	0.81	0.92
P-52	55	55	2.8	1.9	0.71	098	1.16	0.89
P-53	31	61	0.7	1.9	0.40	1.02	0.39	1.11
P-71	55	45	3.1	1.4	1.17	0.52	0.87	0.53
P-77	33	64	1.1	3.7	0.39	1.19	0.98	0.91
Pythium G1								
P-119	25	49	0.8	2.8	0.31	0.94	0.40	0.72
P-217	39	39	1.4	1.9	0.55	0.70	1.02	0.52
P-221	4.6	14	0.3	0.6	011	0.21	0.17	0.35
Pythium G2								
P-9	39	58	1.5	2.6	0.62	1.04	0.64	1.05
P-11	33	48	0.9	2.0	0.42	0.72	0.75	0.78
P-41	35	64	0.7	3.7	0.72	1.16	0.99	0.88
P-90	62	70	3.6	3.4	1.24	1.27	1.51	1.16
P-110	4.6	56	0.1	3.3	0.02	1.03	0.27	0.52
Pythium G3								
P-15	63	59	3.5	2.3	1.23	0.96	1.04	0.71
P-111	49	69	2.3	3.8	0.92	1.42	0.79	1.26
Pythium G4								
P-48	68	68	4.8	3.1	1.48	1.19	1.07	1.24
Control	81	90	6.3	4.9	1.58	1.66	1.03	1.28
LSD ($P \leq 0.05$)	17	20	0.97	1.32	0.320	0.406	0.363	0.354

[z]Ten seeds per pot, ten replicate pots per treatment. Percent seedling survival and plant height recorded 2 wk after seeding,and shoot and root dry weights determined 1 month after seeding.

Table 2. Linear regression of inoculation concentration of *Pythium* spp. on seedling survival of peas.

Days after inoculation	Express		Radley	
	Equation	R2 (%)	Equation	R2 (%)
Day 7	y = 82.0 – 14.1x	92.1**	y = 71.2 – 12.4x	84.8**
Day 10	y = 85.4 – 14.2x	90.3**	y = 79.9 – 14.6x	93.2**
Day 12	y = 84.9 – 14.0x	89.7**	y = 81.6 – 15.0x	93.9**
Day 14	y = 85.4 – 14.0x	90.1**	y = 83.0 – 15.2x	94.3**
Day 17	y = 84.9 – 14.0x	90.0**	y = 84.6 – 15.5x	93.3**
Day 19	y = 85.1 – 14.1x	90.5**	y = 84.8 – 15.6x	93.3**
Day 21	y = 85.9 – 14.2x	91.2**	y = 84.9 – 15.5x	93.5**
Day 26	y = 86.7 – 14.3x	90.7**	y = 85.0 – 15.4x	93.5**

Linear regression parameters calculated after square root transformation of inoculum densities.
** significant at $P = 0.01$.

Table 3. Average effect of seed treatment with metalaxyl on field pea seedling survival and growth in soil infested with six isolates of *Pythium* spp. in a greenhouse trial[z].

	Survival (%)		Plant height (cm)		Shoot dry wt. (g)		Root dry wt. (g)	
Pythium								
Isolate	M[†]	C	M	C	M	C	M	C
P. irregulare								
P-31	83.2	46.8	4.9	3.8	1.3	0.6	0.8	0.5
P. ultimum								
P-113	80.1	56.2	5.0	4.3	1.3	0.8	1.0	0.7
Pythium G1								
P-119	81.0	47.2	5.1	4.1	1.2	0.6	0.9	0.7
P-217	79.0	56.1	4.9	3.9	1.2	0.7	0.9	0.7
P-221	86.9	39.0	4.6	3.7	1.2	0.4	0.9	0.5
Pythium G2								
P-110	82.3	36.6	5.6	3.3	1.3	0.4	0.8	0.5
LSD ($P \leq 0.05$)	ns	12.3	0.9	0.7	ns	0.2	0.1	0.1

[z]Ten seeds per pot, six pots per treatment. Percent seedling survival and plant height recorded 2 weeks after seeding, and shoot and root dry weights measured 1 month after seeding.

* Means in a column within each group followed by a common letter are not significantly different at $P \leq 0.05$.

[†]M= Metalaxyl; C= Control

Table 4. Analysis of variance of the effect of two Apron seed treatments on number of emerged seedlings and seed yield of field pea in Brooks and Vegreville, Alberta in 1997 – 1998.

Source of variance	df	Mean squares	
		Seedling #	Yield
Brooks:			
Replication	3	138.1	206437.1**
Cultivar	1	1252.6**	285686.5*
Year	1	3626.2**	65734.2
Treatment	2	1864.7**	558971.6**
Cultivar x year	1	126.1	5388877.2**
Year x treatment	2	948.5**	174447.4
Cultivar x treatment	2	71.5	10567.8
Cultivar x year x treatment	2	175.2	95167.9
Vegreville:			
Replication	3	33.0	71095.4
Cultivar	1	3614.0**	89839.8
Year	1	728.5**	1552749.4**
Treatment	2	706.5**	231127.5**
Cultivar x year	1	13.5	80557.7
Year x treatment	2	117.4*	7412.4
Cultivar x treatment	2	39.2	1558.6
Cultivar x year x treatment	2	15.6	80705.0

Table 5. Effects of two Apron seed treatments on number of emerged seedlings and seed yield of field pea in Brooks and Vegreville, Alberta in 1997 – 1998.

	Seedling # (6 m row)		Yield (kg/ha)	
	Brooks	Vegreville	Brooks	Vegreville
Treatment:				
Apron XL LS	66.0 a	54.8 a	2026.0 a	1193.9 a
Apron FL	66.7 a	54.6 a	2077.9 a	1188.1 a
None	47.6 b	43.2 b	1731.3 b	982.9 b
Cultivar:				
Carneval	65.2 a	59.6 a	1867.9 b	1164.9 a
Carrera	55.0 b	42.2 b	2022.2 a	1078.4 a
Year:				
1997	68.8 a	47.0 b	1982.1 a	1301.5 a
1998	51.4 b	54.8 a	1908.1 a	941.8 b

* Means in a column within each group followed by a common letter are not significantly different at $P \leq 0.05$.

Table 6. Analysis of variance of the effect of seed damage and seed treatment with metalaxyl on seedling survival and growth of field pea cv. Carneval in cool (10/20 °C) and cold (6/12 °C) soils.

Source of variance	df	Mean square			
		Seedling survival	Shoot height	Shoot dry weight	Root rot severity
Replication	8	249.4	8.3	0.05	0.3
Treatment	3	10454.1**	227.3**	1.70**	14.9**
Error A	24	173.5	3.0	0.02	0.3
Temperature	1	0.1	757.5**	6.70**	9.8**
Treatment x temperature	3	1600.6**	59.7**	0.56**	2.1**
Error B	8	225.2	7.6	0.05	0.3
Total error	96	166.8	2.8	0.02	0.3

Table 7. Analysis of variance of the effect of seed treatment and mechanical damage on seedling emergence and yield of pea in field trials, 1996 – 1998.

Source of variance	df	Mean square: Seedling # (5 m)	Mean square: Yield (g/5 m^2)
Vegreville, 1996:			
Replication	3	15.4	114579.3
Cultivar	1	97.2*	8172267.6**
Error A	3	8.4	67651.2
Inoculation	1	43.2	96697.8
Cultivar x inoculation	1	0.1	118357.2*
Error B	3	8.4	86078.1
Treatment	5	991.7**	383443.1**
Inoculation x treatment	5	5.6	15404.6
Cultivar x treatment	5	165.6*	19016.1
Cultivar x inoculation x treatment	5	2.6	20372.7
Error C	15	7.4	23322.5
Total Error	48	7.0	28161.6
Brooks, 1997:			
Replication	3	78.2**	2550624.2
Cultivar	1	149.4**	372.1
Error A	3	2.3	445940.7
Inoculation	1	7.2	316136.3
Cultivar x inoculation	1	49.2	34088.3
Error B	3	16.1	255008.2
Treatment	5	622.3**	389890.5**
Inoculation x treatment	5	14.3	45241.8
Cultivar x treatment	5	22.9	71784.6
Cultivar x inoculation x treatment	5	12.4	112838.8
Error C	15	13.3	60112.0
Total Error	48	17.5	91963.9
Namao, 1998:			
Replication	3	27.0	933.7
Cultivar	1	281.6**	1775356.4**
Error A	3	2.0	4448.6
Inoculation	1	583.5**	324273.3**
Cultivar x inoculation	1	8.1	1844.7
Error B	3	16.3	1728.2
Treatment	3	161.3**	138000.0**
Inoculation x treatment	3	30.4*	780.3
Cultivar x treatment	3	5.5	9087.7
Cultivar x inoculation x treatment	3	4.7	2167.5
Error C	9	20.8	6660.9
Total Error	30	9.0	5550.5

Table 8. The effect of seed treatment and mechanical damage on seedling emergence and yield of pea in field trials, 1996 – 1998.

Seed treatment		Seedling # (5 m)			Yield (g/5 m^2)		
		1996	1997	1998	1996	1997	1998
Damage type:							
None	Yes	44.5 a	44.8 a	48.3 a	1295.9 a	1995.7 a	1474.2 a
None	No	33.2 d	38.0 b	43.5 b	1107.9 b	1900.0 ab	1335.4 b
Drop	Yes	39.7 c	32.4 c	48.1 a	1258.7 a	1665.6 c	1381.5 b
Drop	No	29.6 e	28.7 d	42.1 b	1014.8 bc	1634.4 c	1251.6 c
Auger	Yes	42.1 b	34.6 c	--	1250.3 a	1719.4 bc	--
Auger	No	24.4 f	28.3 d	--	910.7 c	1616.9 c	--
Cultivar:							
Carneval		34.6 b	35.7 a	47.6 a	1431.5 a	1753.4 a	1527.2 a
Montana		36.6 a	33.2 b	43.4 b	848.0 b	1757.3 a	1194.1 b
Inoculation:							
Inoculated		34.9 a	34.7 a	42.5 b	1108.0 a	1697.9 a	1289.5 b
Non-inoculated		36.2 a	34.2 a	48.5 a	1171.5 a	1812.7 a	1431.9 a

• Means in a column followed by a common letter are not significantly different at $P \leq 0.05$.

List of Figures

Figure 1A. Linear regression of inoculum concentration of *Pythium* spp. on seedling shoot dry weight of pea cvs. Express and Radley.

Figure 1B. Linear regression of inoculum concentration of *Pythium* spp. on seedling root dry weight of pea cvs. Express and Radley.

Figure 2. Effect of seed damage and seed treatment on seedling survival, shoot height and dry weight, and root rot severity under controlled conditions at two temperatures.